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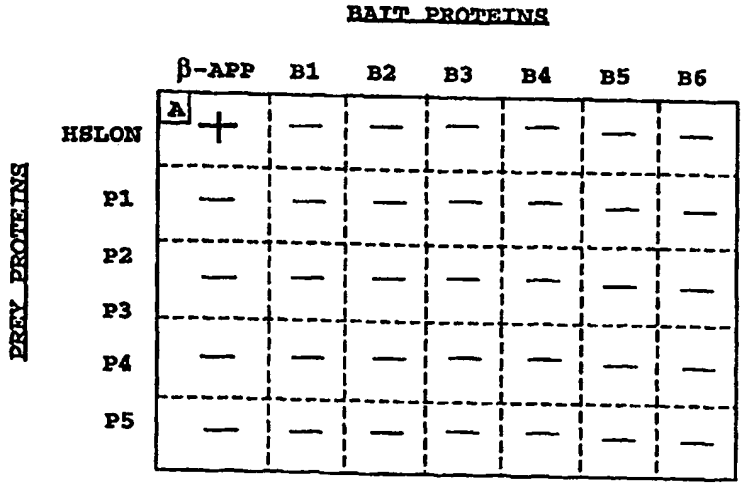

(57) Abstract

The present invention discloses an interaction between β–APP and HsLON and the formation of a β–APP:HsLON complex, or of the derivatives, fragments, analogs and homologs thereof, that were identified using a modified, improved yeast two hybrid assay system. Methodologies of screening these aforementioned complexes for efficacy in treating and/or preventing various diseases and disorders, particularly neurodegenerative disease, cardiomyopathy, diabetes, hearing loss, male infertility, mitochondrial DNA mutation associated disorders and the like, are also disclosed herein.

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INTERACTION OF HUMAN BETA AMYLOID PRECURSOR PROTEIN (β-APP) WITH HUMAN LON-PROTEASE LIKE PROTEIN (HSLON)

GRANT SUPPORT

This invention was made with United States Government support under grant number 70NANB5H1066 awarded by the National Institute of Standards and Technology. Accordingly, the United States Government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention is directed to complexes of beta-APP and HsLON proteins. In addition, the present invention relates to the production of antibodies to the aforementioned protein complex, and its use in, *inter alia*, screening, diagnosis, prognosis and therapy.

BACKGROUND OF THE INVENTION

1. β-APP

The human beta amyloid precursor (β-APP) protein has been the subject of intense study since amyloid plaques containing fragments of the β-APP protein represent a key pathophysiological feature in the brains of patients with Alzheimer's Disease (AD) (Glenner and Wong, 1984, Biochem. Biophys. Res. Commun. 120:885-890). The first mRNA for β-APP was cloned in 1988 (GenBank Accession No. A02759; Mueller-Hill *et al.*, 1988, European Patent Publication EP 276723). This mRNA encodes a neuronally-expressed protein of 695 amino acids. Three other protein precursors of 714, 751, and 770 amino acids are also expressed via differential splicing (Kang and Muller-Hill, 1990, Biochem. Biophys. Res. Commun 166:1192-1200).

A main focus of AD research has been the possibility that abnormal processing of β-APP results in products that collect in the brain and form damaging deposits. The principal product in amyloid plaques is β-A4, a 39-42 residue fragment of β-APP located at the start of the C-terminal third of the precursor molecule (Kang *et al.*, 1987, Nature 325:733-736). While this fragment is a normally expressed product in human brain, its accumulation is greatly accelerated in some disorders including AD and trisomy 21 (Suebert *et al.*, 1992, Nature 359:325-327). Mutations in the β-APP gene seem to account for a small percentage of AD cases, and thus, abnormalities of other factors, such as processing enzymes, cofactors, etc., involved in β-A4 folding, stability, or targeting are also likely to account for accelerated accumulation and deposition of β-A4 (Seubert *et al.*, 1993, Nature 361:260-263).

The apolipoprotein E (apoE) protein associates with β-A4. ApoE may act as a pathological molecular chaperone that binds to soluble β-A4 and stabilizes abnormal β-pleated sheet formation, and thus, plaque formation (Wisniewski *et al.*, 1992, Neurosci. Lett. 135:235-238). Finally, the presenilin proteins PS-1 and PS-2 display a linkage with some cases of early-onset familial AD (Sherrington *et al.*, 1995, Nature 375:754-760; Cruts *et al.*, 1996, Hum. Mol. Genet. 5:1449-1455). The results of the above-mentioned studies indicate that abnormal protein-protein interactions of, or functioning of, β-A4, or other products of β-APP, may underlie the pathogenesis of some or all forms of AD.

Mitochondrial dysfunction may also be involved in the pathogenesis of AD. A mutation in codon 331 of mitochondrially-encoded subunit 2 of NADH dehydrogenase (complex I of the oxidative phosphorylation chain) has been reported in 10/19 AD patients, and 2/6 patients with amyotrophic lateral sclerosis (ALS) (Lin *et al.*, 1992, Biochem. Biophys. Res. Commun. 182:238-246). Thus, mitochondrial damage is associated with neurodegenerative diseases. A unifying hypothesis regarding the pathogenesis of AD has been formed around the central theme of increased oxidative damage to mitochondria causing disrupted calcium homeostasis and neuronal degeneration (Markesbery, 1997, Free Radic. Biol. Med. 23:134-147; Meier-Ruge and Bertoni-Freddari, 1996, Rev. Neurosci. 7:1-19). Assembly of inner mitochondrial membrane respiratory complexes occurs by addition of mitochondrially-encoded subunits around cores of imported protein subunits (Hall and Hare, 1990, J. Biol. Chem. 265:16484-16490). If assembly is delayed, free mitochondrially-encoded subunits are rapidly degraded in an oligomycin-sensitive manner, thus implicating an ATPase-dependent protease as important to mitochondrial biogenesis, and thus, protection against neurodegenerative disease (Meier-Ruge and Bertoni-Freddari, 1996, Rev. Neurosci. 7:1-19). Thus, mitochondrial dysfunction and the protein components involved therein are implicated along with abnormal β-APP function in the pathogenesis of AD.

2. HsLON

The human Lon-protease-like (HsLON) protein (GenBank Accession No. X74215; Amerik *et al.*, 1994, FEBS Lett. 340:25-28) is the homologue of Lon, an *E. coli* protease which is involved in the degradation of misfolded proteins. In yeast, the mitochondrially-localized protease PIM1 is homologous to Lon, and PIM1 and HsLON function interchangeably in yeast to degrade misfolded proteins in the mitochondrial matrix (Suzuki *et al.*, 1994, Science 264:273-276; Teichmann *et al.*, 1996, J. Biol. Chem. 271:10137-10142). Yeast expressing mutant *PIM1* display aberrant assembly of inner mitochondrial membrane respiratory complexes, become respiratory deficient, and ultimately suffer lost integrity of their mitochondrial DNA. Like the yeast homologue, human LON (HsLON) is an ATPase-dependent protease that can function in mitochondrial biogenesis and maintenance (Suzuki *et al.*, 1997, Trends Biochem. Sci. 22:118-123).

HsLON is implicated in all aspects of mitochondrial maintenance and function. As such, it can affect physiological processes including, but not limited to, mitochondrial biogenesis, cell oxidative capacity and energy metabolism, cell apoptosis, calcium homeostasis, and the pathological processes of Alzheimer's Disease (AD), dementia of trisomy 21, amyotrophic lateral sclerosis (ALS), and other neurodegenerative disorders. Further, mutations in mitochondrial DNA, which increase both with age and oxidative stress, are associated with a wide range of both spontaneous and heritable clinical disorders (Odawara, 1996, New Engl. J. Med. 334:270-271; Hammans, 1994, Essays Biochem. 28:99-112). In many, but not all cases, these mutations involve a point mutation at a point mutation "hot spot", (for example, C3254G or A3243T in the mitochondrial tRNA(Leu) gene). However, most disorders are also seen with more generalized mitochondrial DNA deletions (Rose, 1998, Arch. Neurol. 55:17-24).

Most of the disorders involve myopathies caused by underlying mitochondrial dysfunction. Among these are MELAS syndrome (Mitochondrial myopathy, Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes; Saitoh *et al.*, 1998, Neurology, 50:531-534), progressive external ophthalmoplegia (Melberg *et al.*, 1998, Neurology, 50:299-300), both autosomal and non-autosomal cardiomyopathies (Takeda *et al.*, 1996, Diabetes Res. Clin. Pract. 31 Suppl. S123-126), and some forms of polymyalgia rheumatica (Reynier *et al.*, 1994, Biochem. Biophys. Res. Commun. 205:375-380). Many mitochondrial DNA mutations are also associated with CNS degeneration, including Parkinson's disease (Ozawa *et al.*, 1991, Biochem. Biophys. Res. Comm. 177:518-525), Alzheimer's Disease, including morphologically-identified amyloid plaques (Kaido *et al.*, 1996, Acta Neuropath. [Berlin] 92:312-318), and late-onset encephalopathy (Johnston *et al.*, 1995, Ann. Neurol. 37:16-23). Thus, evidence exists that mitochondrial dysfunction, either resulting from mitochondrial DNA mutations, or mitochondrial dysfunction associated with aging, oxidative stress, etc., is associated with neurodegenerative disease.

Mitochondrial DNA mutations also underlie diseases and disorders such as MERRF syndrome (Myoclonic Epilepsy with Ragged Red Fibers; Blumenthal *et al*, 1998, Neurology 50:524-525), multiple symmetric lipomatosis (Klopstock *et al.*, 1997, Mol. Cell. Biochem. 174:271-275), Kearns-Sayre syndrome (pigmented retinopathy, bilateral progressive ophthalmoplegia, and cardiac conduction defects; Zoviani *et al.*, 1988, Neurology 38:1339-1346), male infertility (St. John *et al.*, 1997, Nat. Med. 3:124-125), sensorineural hearing loss (Donovan *et al.*, 1995, Ann. Otol. Rhinol. Laryngol. 104:786-792), maternally-inherited deafness (Silvestri *et al.*, 1994, Human Mutat. 3:37-43), and maternally-inherited diabetes (Tsukada *et al.*, 1997, Diabetes Med. 14:1032-1037).

In summary, besides involvement in neurodegenerative disease, HsLON may also be implicated in cardiomyopathy, some forms of diabetes, hearing loss, male infertility, and a number of less common syndromes and disorders associated with mitochondrial DNA mutations.

To conclude, both β-APP and HsLON are implicated in neurodegenerative processes and mitochondrial function. As such, each or both can affect physiological processes including, but not limited to, mitochondrial biogenesis, cell oxidative capacity and energy metabolism, cell apoptosis, calcium homeostasis, and pathological processes including, but not limited to, neurodegenerative disorders such as Alzheimer's Disease (AD), dementia of trisomy 21, Parkinson's disease, amyotrophic lateral sclerosis (ALS); cardiomyopathy; some forms of diabetes; hearing loss; and male infertility; as well as a number of less common syndromes and disorders that are associated with mitochondrial DNA mutations.

β-APP and HsLON have been described to be involved in similar processes, however no direct association or interaction of β-APP with HsLON has been described previously to the present invention.

Citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

SUMMARY OF THE INVENTION

The present invention is based, in part, on the discovery of a novel interaction between β-amyloid precursor protein (β-APP) and the HsLON protein, in particular, interaction between the predominantly expressed 695 amino acid form of β-APP, (to which the term β-APP refers in this Application), or the portion of β-APP comprised of residues 597-638, (herein referred to as β-A4), and HsLON, or the C-terminal 126 residues of HsLON.

The present invention is directed to certain compositions comprising and methods for production of protein complexes of β-APP with a protein that interacts with (*i.e.*, binds to) β-APP. Specifically, the invention is directed to complexes of β-APP, and derivatives, fragments and analogs of β-APP, with HsLON, and its derivatives, fragments and analogs (a complex of β-APP and HsLON is designated as β-APP:HsLON herein). The present invention is further directed to methods of screening for proteins that interact with β-APP and/or HsLON, or with derivatives, fragments or analogs of β-APP and/or HsLON.

Methods for production of the β-APP:HsLON complex, and derivatives and analogs of the complex and/or individual proteins, *e.g.*, by recombinant means, are also provided. Pharmaceutical compositions are also provided.

The invention is further directed to methods for modulating (*i.e.*, inhibiting or enhancing) the activity of a β-APP:HsLON complex. The protein components of a β-APP:HsLON complex have been implicated in physiological processes including, but not limited to, mitochondrial biogenesis, cell oxidative capacity and energy metabolism, cell apoptosis, calcium homeostasis, and pathological processes including, but not limited to, neurodegenerative disorders such as Alzheimer's Disease, dementia of trisomy 21, Parkinson's disease, amyotrophic lateral sclerosis (ALS); cardiomyopathy; some forms of diabetes; hearing loss; and male infertility; as well as a number of less common syndromes and disorders associated with mitochondrial DNA mutations. Accordingly, the present invention is directed to methods for screening β-APP:HsLON complexes, as well as derivatives and analogs of the β-APP:HsLON complex, for the ability to alter a cell function, particularly a cell function in which β-APP and/or HsLON has been implicated, as non-exclusively listed, *supra*.

The present invention is also directed to therapeutic and prophylactic, as well as diagnostic, prognostic, and screening methods and compositions based upon the β-APP:HsLON complex (and the nucleic acids encoding the individual proteins that participate in the complex). Therapeutic compounds of the invention include, but are not limited to, a β-APP:HsLON complex, and a complex where one or both members of the complex is a derivative, fragment, homolog or analog of β-APP or HsLON; antibodies to and nucleic acids encoding the foregoing; and antisense nucleic acids to the nucleotide sequences encoding the complex components. Diagnostic, prognostic and screening kits are also provided.

Animal models and methods of screening for modulators (*i.e.*, agonists, and antagonists) of the activity of a β-APP:HsLON complex are also provided.

Methods of identifying molecules that inhibit, or alternatively, that increase formation of a β-APP:HsLON complex are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. The nucleotide sequence of β-APP (GenBank Accession No. A02759 (SEQ ID NO: 1) and deduced amino acid sequence (SEQ ID NO:2). The coding sequence beginning at base 1935 (amino acid 597), marked by arrow "A", and ending at base 2060 (amino acid 638), marked by arrow "B", was used as bait in the assays described *infra*.

Figure 2. The nucleotide sequence (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of human HsLON (GenBank Accession No. X74215). The 5' start site of the identified prey sequence was at base 2194 (amino acid 721), marked by arrow "A".

Figure 3. Demonstration of the specificity of the β-APP:HsLON interaction. A positive interaction for a bait and prey proteins is indicated as "+" in the box forming the intersection between the particular bait and prey proteins, a lack of interaction is designated by "-". The intersection of the β-APP column with the HsLON row indicates growth (Box A) (*i.e.*, a positive interaction). In contrast, the intersection of the β-APP column with the rows for P1–P5, and the intersection of the HsLON row with columns for B1–B6, indicates no growth, *i.e.*, no protein interaction. These data demonstrate the specificity of the β-APP:HsLON interaction.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based, in part, upon identification of proteins that interact with β-APP (SEQ ID NOS:2) using a modified form of the yeast matrix mating test. At least amino acids 721 to 845 of HsLON (SEQ ID NOS:4) were found to form a complex under physiological conditions with at least amino acids 597 to 638 of β-APP (SEQ ID NOS:2) (the complex of β-APP with HsLON is indicated as " β-APP:HsLON" herein). The β-APP:HsLON complex, by virtue of the interaction, is implicated in modulating the functional activities of β-APP and its binding partner. Such functional activities include physiological processes including, but not limited to, neurodegenerative disorders such as Alzheimer's Disease, dementia of trisomy 21, Parkinson's disease, amyotrophic lateral sclerosis (ALS); cardiomyopathy; some forms of diabetes; hearing loss; and male infertility; as well as a number of less common syndromes and disorders associated with mitochondrial DNA mutations.

The present invention is also directed to methods of screening for proteins that interact with, *e.g.*, bind to β-APP. The present invention further discloses a complex of the β-APP protein, or a derivative, analog or fragment thereof, in particular with HsLON protein, or a derivative, analog or fragment thereof. In a preferred embodiment, such complex binds an anti-β-APP, anti-HsLON, and/or anti-β-APP:HsLON complex antibody. In another specific embodiment of the present invention, a complex of human β-APP with human HsLON is provided.

The present invention also provides methodologies for the production and/or isolation of β-APP:HsLON complex. In a specific embodiment, the present invention provides methodology of using recombinant DNA techniques to express both β-APP and HsLON (or a derivative, fragment or analog of one or both members of the complex) wherein both binding partners are under the control of one heterologous promoter (*i.e.*, a promoter not naturally associated with the gene encoding the particular complex component) or where each is under the control of a separate heterologous promoter.

Methods of diagnosis, prognosis, and screening for diseases and disorders associated with aberrant levels of a β-APP:HsLON complex are discloses. The present invention also provides

methodology for the treatment or prevention of diseases or disorders associated with an aberrant level of β-APP:HsLON complex, or an aberrant level of activity of one or more of the components of the complex, by administration of a β-APP:HsLON complex, or modulators of β-APP:HsLON complex activity or formation (*e.g.*, antibodies that bind to a β-APP:HsLON complex), or non-complexed β-APP or its binding partner or a derivative, fragment, analog or homolog thereof. Preferably, the aforementioned derivative, fragment, analog or homolog contains: (i) the portion of β-APP or HsLON that is directly involved in complex formation; (ii) mutants of β-APP or of HsLON that modulate binding affinity; (iii) small molecule inhibitors or enhancers of complex formation; or (iv) antibodies that either stabilize or neutralize the complex, and the like.

Methodologies of assaying β-APP:HsLON complex for biological activity as a therapeutic or diagnostic, as well as methodologies for screening for β-APP:HsLON complex or modulators thereof (*i.e.*, agonists and antagonists), are also disclosed herein.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the following subsections.

(1) The β-APP protein, the HsLON protein and the β-APP:HsLON complex

The present invention discloses the complex of β-APP with HsLON (the β-APP:HsLON complex). In a preferred embodiment, the β-APP:HsLON complex is a complex of human proteins. The present invention also relates to: (*i*) complexes of derivatives, fragments and analogs of the β-APP with a HsLON; (*ii*) complexes of the β-APP with derivatives, fragments and analogs of HsLON and (*iii*) complexes of derivatives, fragments and analogs of the β-APP and HsLON. It should be noted that, as used herein, fragment, derivative or analog of a β-APP:HsLON complex includes complexes where one or both members of the complex are fragments, derivatives or analogs of the wild-type β-APP or HsLON protein.

Derivatives, fragments, and analogs provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively. Fragments are, at most, one nucleic acid-less or one amino acid-less than the wild type full length sequence. Derivatives and analogs may be full length or other than full length, if said derivative or analog contains a modified nucleic acid or amino acid, as described *infra*. Derivatives or analogs of β-APP and HsLON include, but are not limited to, molecules comprising regions that are substantially homologous to β-APP or HsLON, in various embodiments, by at least about 30%, 50%, 70%, 80%, or 90% identity (with a preferred identity of 80-95%) over an amino acid sequence of

identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, for *e.g.*, Wisconsin GCG (Genetics Computer Group) software package using default parameter settings. Alternatively, comparison may be made to an aligned sequence whose encoding nucleic acid is capable of hybridizing to the complement (*e.g.*, the inverse complement) of a sequence encoding β-APP or HsLON under stringent (the preferred embodiment), moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and *infra*.

Preferably, as disclosed by the present invention, the β-APP:HsLON complex in which one or both members of the complex are a fragment, derivative or analog of the wild-type protein are functionally active β-APP:HsLON complex. In particular aspects, the native proteins (or homologs, fragments, derivatives or analogs thereof) of the β-APP and/or HsLON are of insects (*e.g.*, fly), plants, or animals (*e.g.*, mouse, rat, pig, cow, dog, monkey, frog) or, most preferably, human. As utilized herein, the term "functionally active β-APP:HsLON complex" refers to species displaying one or more known functional attributes of a full-length β-APP (or derivatives, fragments, analogs or homologs thereof), complexed with full-length HsLON (or derivatives, fragments, analogs or homologs thereof), including, but not exclusive to, the control of cell cycle progression, cellular differentiation and apoptosis, intracellular signal transduction, neurogenesis, response to viral infection, a hyperproliferative disorder such as tumorigenesis and tumor spread, a degenerative disorder such as a neurodegenerative disease, autoimmune disease, a disorder associated with organ transplantation, inflammatory and/or allergic disease, atherosclerosis, nephropathy, cardiac disease, muscle disease, or the like.

Specific embodiments of the present invention disclose the β-APP:HsLON complex comprised of fragments of one or both protein species of the complex. In a preferred embodiment, these aforementioned fragments may consist of, but are not limited to, fragments of HsLON that have been identified as interacting with the β-APP in an improved, modified yeast two hybrid assay in this invention, *i.e.*, amino acids 721-845 of HsLON as depicted in Figure 2 [SEQ ID NO:4]. In addition, fragments (or proteins comprising fragments) that may lack some or all of the aforementioned regions of either member of the complex, as well as nucleic acids that encode the aforementioned proteins, are also disclosed herein.

The nucleotide sequences encoding human β-APP and human HsLON are known, (GenBank Accession No. A02759 and GenBank Accession No. X74215, respectively), and are disclosed in Figures 1 and 2 (SEQ ID NOS:1 and 3, respectively). Nucleic acids may be obtained by any method known within the art (*e.g.*, by PCR amplification using synthetic primers hybridizable to the 3'- and 5'-termini of the sequence and/or by cloning from a cDNA or genomic library using an oligonucleotide sequence specific for the given gene sequence, or the like).

Homologs (*i.e.*, nucleic acids encoding the aforementioned proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can also be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

In a most preferred embodiment, a nucleic acid sequence that is hybridizable to a nucleic acid sequence (or a complement of the foregoing) encoding β-APP and/or HsLON, or a derivative of the same, under conditions of high stringency is provided. By way of example and not limitation, procedures using such conditions of high stringency are as follows: Step 1: Filters containing DNA are pretreated for 8 hours to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 μg/ml denatured salmon sperm DNA. Step 2: Filters are hybridized for 48 hours at 65°C in the above prehybridization mixture to which is added 100 μg/ml denatured salmon sperm DNA and 5-20 x 106 cpm of ^{32}P-labeled probe. Step 3: Filters are washed for 1 hour at 37°C in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 minutes. Step 4: Filters are autoradiographed. Other conditions of high stringency that may be used are well known in the art. See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a second embodiment, a nucleic acid sequence that is hybridizable to a nucleic acid sequence (or a complement of the foregoing) encoding β-APP and/or HsLON, or a derivative of either, under conditions of moderate stringency is provided. By way of example and not limitation, procedures using such conditions of moderate stringency are as follows: Step 1: Filters containing DNA are pretreated for 6 hours at 55°C in a solution containing 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 μg/ml denatured salmon sperm DNA. Step 2: Filters are hybridized for 18-20 hours at 55°C in the same solution with 5-20 x 106 cpm ^{32}P-labeled probe added. Step 3: Filters are washed at 37°C for 1 hour in a solution containing 2X SSC, 0.1% SDS, then washed twice for 30 minutes at 60°C in a solution containing 1X SSC and 0.1% SDS. Step 4: Filters are blotted dry and exposed for autoradiography. Other conditions of moderate stringency that may be used are well-known in the art. See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to a β-APP and/or HsLON nucleic acid sequence or to a nucleic acid sequence encoding a β-APP and/or HsLON derivative (or a complement of the foregoing), under conditions of low stringency, is provided. By way of example and not limitation, procedures using such conditions of low stringency are as follows (see also Shilo and Weinberg, 1981, *Proc. Natl. Acad. Sci. USA 78*: 6789-6792): Step 1: Filters containing DNA are pretreated for 6 hours at

40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 µg/ml denatured salmon sperm DNA. Step 2: Filters are hybridized for 18-20 hours at 40°C in the same solution with the addition of 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 x 106 cpm ^{32}P-labeled probe. Step 3: Filters are washed for 1.5 hours at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 hours at 60°C. Step 4: Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency that may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations). See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

The invention also relates to nucleic acids hybridizable to or complementary to the foregoing sequences, in particular the invention provides the inverse complement to nucleic acids hybridizable to the foregoing sequences (*i.e.*, the inverse complement of a nucleic acid strand has the complementary sequence running in reverse orientation to the strand so that the inverse complement would hybridize with little or no mismatches to the nucleic acid strand). In specific aspects, nucleic acid molecules are provided that comprise a sequence complementary to (specifically, are the inverse complement of) at least about 10, 25, 50, 100, or 200 nucleotides or the entire coding region of a β-*APP* and/or *HsLON* gene (SEQ ID NOS:1 and 3, respectively). Nucleic acid molecules encoding derivatives and analogs of β-APP and/or HsLON (*supra*), or antisense nucleic acids to the same (see, *e.g.*, *infra*) are additionally provided.

Within nucleotide sequences encoding polypeptides identified as beta APP interactants via the modified yeast two hybrid assay in this invention, potential open reading frames can be identified using the NCBI BLAST program ORF Finder available to the public. Because all known protein translation products are at least 60 amino acids or longer (Creighton, 1992, PROTEINS, 2nd Ed., W.H. Freeman and Co., New York), only those ORFs potentially encoding a protein of 60 amino acids or more are considered. If an initiation methionine codon (ATG) and a translational stop codon (TGA, TAA, or TAG) are identified, then the boundaries of the protein are defined. Other potential proteins include any open reading frames that extend to the 5' end of the nucleotide sequence, in which case the open reading frame predicts the C-terminal or core portion of a longer protein. Similarly, any open reading frame that extends to the 3' end of the nucleotide sequence predicts the N-terminal portion of a longer protein.

Recombinant Technologies for obtaining the complex or β-APP or HsLON

The β-APP and HsLON protein, either alone or within a complex, may be obtained by methods well-known in the art for protein purification and recombinant protein expression. For recombinant expression of one or more of the proteins, the nucleic acid containing all or a portion of the nucleotide sequence encoding the protein may be inserted into an appropriate expression vector (*i.e.*, a vector that contains the necessary elements for the transcription and translation of the inserted protein coding sequence). In a preferred embodiment, the regulatory elements are heterologous (*i.e.*, not the native gene promoter). Alternately, the necessary transcriptional and translational signals may also be supplied by the native promoter for the β-APP or any HsLON genes and/or their flanking regions.

A variety of host-vector systems may be utilized to express the protein coding sequence(s). These include, but are not limited to: (*i*) mammalian cell systems that are infected with vaccinia virus, adenovirus, and the like; (*ii*) insect cell systems infected with baculovirus and the like; (*iii*) yeast containing yeast vectors or (*iv*) bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

In a preferred embodiment, the β-APP:HsLON complex are obtained by expressing the entire β-APP coding sequence and a HsLON coding sequence within the same cell, either under the control of the same promoter or two separate promoters. In another embodiment, a derivative, fragment or homolog of the β-APP and/or a derivative, fragment or homolog of a HsLON are recombinantly expressed. Preferably, the derivative, fragment or homolog of the β-APP and/or the HsLON protein form a complex with a binding partner that has been identified by a binding assay (*e.g.*, the modified yeast two hybrid system assay) and, more preferably, form a complex that binds to an anti-β-APP, anti-HsLON and/or anti-β-APP:HsLON complex antibody.

Any of the methodologies known within the relevant prior art regarding the insertion of nucleic acid fragments into a vector may be utilized to construct expression vectors that contain a chimeric gene comprised of the appropriate transcriptional/translational control signals and protein-coding sequences. These methodologies may include, but are not limited to, *in vitro* recombinant DNA and synthetic techniques, as well as *in vivo* recombination techniques (*e.g.*, genetic recombination). The expression of nucleic acid sequences that encode the β-APP and the HsLON protein, or derivatives, fragments, analogs or homologs thereof, may be regulated by a second nucleic acid sequence such that the genes or fragments thereof are expressed in a host that has been concomitantly transformed with the recombinant DNA molecule(s) of interest. The expression of the specific proteins may be controlled by any promoter/enhancer known in the art including, but not limited to: (*i*) the SV40 early promoter (see *e.g.*,

Bernoist & Chambon, 1981. *Nature* 290: 304-310); (*ii*) the promoter contained within the 3'-terminus long terminal repeat of Rous Sarcoma Virus (see *e.g.*, Yamamoto, *et al.*, 1980. *Cell* 22: 787-797); (*iii*) the Herpesvirus thymidine kinase promoter (see *e.g.*, Wagner, *et al.*, 1981. *Proc. Natl. Acad. Sci. USA* 78: 1441-1445); (*iv*) the regulatory sequences of the metallothionein gene (see *e.g.*, Brinster, *et al.*, 1982. *Nature* 296: 39-42); (*v*) prokaryotic expression vectors such as the β-lactamase promoter (see *e.g.*, Villa-Kamaroff, *et al.*, 1978. *Proc. Natl. Acad. Sci. USA* 75: 3727-3731); (*vi*) the *tac* promoter (see *e.g.*, DeBoer, *et al.*, 1983. *Proc. Natl. Acad. Sci. USA* 80: 21-25) and the like.

In addition, plant promoter/enhancer sequences within plant expression vectors may also be utilized including, but not limited to: (*i*) the nopaline synthetase promoter (see *e.g.*, Herrar-Estrella, *et al.*, 1984. *Nature* 303: 209-213); (*ii*) the cauliflower mosaic virus 35S RNA promoter (see *e.g.*, Garder, *et al.*, 1981. *Nuc. Acids Res.* 9: 2871) and (*iii*) the promoter of the photosynthetic enzyme ribulose bisphosphate carboxylase (see *e.g.*, Herrera-Estrella, *et al.*, 1984. *Nature* 310: 115-120) and the like.

Promoter/enhancer elements from yeast and other fungi (*e.g.*, the Gal4 promoter, the alcohol dehydrogenase promoter, the phosphoglycerol kinase promoter, the alkaline phosphatase promoter), as well as from animal transcriptional control regions, for example, those that possess tissue specificity and have been used in transgenic animals, may be utilized in the production of proteins of the present invention. Transcriptional control sequences derived from animals include, but are not limited to: (*i*) the insulin gene control region active within pancreatic β-cells (see *e.g.*, Hanahan, *et al.*, 1985. *Nature* 315: 115-122); (*ii*) the immunoglobulin gene control region active within lymphoid cells (see *e.g.*, Grosschedl, *et al.*, 1984. *Cell* 38: 647-658); (*iii*) the albumin gene control region active within liver (see *e.g.*, Pinckert, *et al.*, 1987. *Genes and Devel.* 1: 268-276; (iv) the myelin basic protein gene control region active within brain oligodendrocyte cells (see *e.g.*, Readhead, *et al.*, 1987. *Cell* 48: 703-712); and (*v*) the gonadotrophin-releasing hormone gene control region active within the hypothalamus (see *e.g.*, Mason, *et al.*, 1986. *Science* 234: 1372-1378), and the like.

In a specific embodiment of the present invention, a vector is utilized that comprises a promoter that is operably-linked to nucleic acid sequences that encode β-APP and/or HsLON, or a fragment, derivative or homolog, thereof, one or more origins of replication, and optionally, one or more selectable markers (*e.g.*, an antibiotic resistance gene). In a preferred embodiment, a vector is utilized that is comprised of a promoter operably-linked to nucleic acid sequences encoding both β-APP and HsLON, one or more origins of replication, and, optionally, one or more selectable markers.

In another specific embodiment, an expression vector contains the coding sequences (or portions thereof) of β-APP and HsLON, either together or separately. The expression vector may be generated by subcloning the aforementioned gene sequences into the *EcoRI* restriction site of each of the three

available pGEX vectors (glutathione S-transferase expression vectors; see *e.g.*, Smith & Johnson, 1988. *Gene* 7: 31-40), thus allowing the expression of products in the correct reading frame.

Expression vectors that contain sequences of interest may be identified by three general approaches: (*i*) nucleic acid hybridization, (*ii*) presence or absence of "marker" gene function and/or (*iii*) expression of the inserted sequences. In a first approach, β-APP and HsLON may be detected by nucleic acid hybridization using probes comprising sequences homologous and complementary to the inserted sequences of interest. In a second approach, the recombinant vector/host system may be identified and selected based upon the presence or absence of certain "marker" functions (*e.g.*, binding to an antibody specific for β-APP, HsLON, or a β-APP:HsLON complex, resistance to antibiotics, occlusion-body formation in baculovirus, and the like) caused by the insertion of the sequences of interest into the vector. In a third approach, recombinant expression vectors may be identified by assaying for the expression of the β-APP concomitantly with expression of HsLON by the recombinant vector.

Once the recombinant β-APP and HsLON molecules have been identified and the complex or individual proteins isolated, and a suitable host system and growth conditions have been established, the recombinant expression vectors may be propagated and amplified in-quantity. As previously discussed, expression vectors or their derivatives that can be used include, but are not limited to, human or animal viruses (*e.g.*, vaccinia virus or adenovirus); insect viruses (*e.g.*, baculovirus); yeast vectors; bacteriophage vectors (*e.g.*, lambda phage); plasmid vectors and cosmid vectors, and the like.

A host cell strain may be selected that modulates the expression of inserted sequences of interest, or modifies or processes expressed proteins encoded by said sequences in the specific manner desired. In addition, expression from certain promoters may be enhanced in the presence of certain inducers in a selected host strain; thus facilitating control of the expression of a genetically-engineered β-APP and/or HsLON. Moreover, different host cells possess characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, phosphorylation, and the like) of expressed proteins. Appropriate cell lines or host systems may thus be chosen to ensure the desired modification and processing of the foreign protein is achieved. For example, protein expression within a bacterial system can be used to produce an unglycosylated core protein; whereas expression within mammalian cells ensures "native" glycosylation of a heterologous protein.

In other specific embodiments, β-APP and/or HsLON (or derivatives, fragments, analogs and homologs thereof) may be expressed as fusion or chimeric protein products comprising the protein joined via a peptide bond to a heterologous protein sequence of a different protein. Such chimeric products may be produced by ligating together appropriate nucleic acid sequences that encode desired

amino acids, said ligation retaining the proper coding frames, and subsequently expressing the chimeric products in a suitable host by methods well known in the art. Alternatively, such a chimeric product can be made by protein synthetic techniques (*e.g.*, by use of a peptide synthesizer).

A specific embodiment of the present invention discloses a chimeric protein comprising a fragment of β-APP and/or HsLON. In another specific embodiment, fusion proteins are provided that contain the interacting domains of β-APP and HsLON (the domains involved in the direct formation of β-APP:HsLON complex) and, optionally, have a heterofunctional reagent (*e.g.*, a peptide linker) that serves to both link the two aforementioned proteins and to promote the interaction of β-APP and HsLON binding domains. These fusion proteins may be particularly useful where the stability of the interaction is desirable (*i.e.*, stability due to the formation of the complex as an intramolecular reaction), for example in production of antibodies specific to β-APP:HsLON complex.

In a specific embodiment of the present invention, the nucleic acids encoding proteins, and proteins consisting of or comprising a fragment of β-APP or HsLON that consists of a minimum of 6 contiguous amino acid residues of β-APP and/or HsLON, are provided herein. In another embodiment, the aforementioned protein fragment is comprised of at least 10, 20, 30, 40, or 50 amino acid residues (and preferably not larger that 35, 100 or 200 amino acid residues) of β-APP or HsLON. Derivatives or analogs of β-APP and HsLON include, but are not limited to, molecules comprising regions that are substantially homologous to β-APP or HsLON in various embodiments, of at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or preferably 95% amino acid identity when: (*i*) compared to an amino acid sequence of identical size; (*ii*) compared to an aligned sequence in that the alignment is done by a computer homology program known within the art or (*iii*) the encoding nucleic acid is capable of hybridizing to a sequence encoding β-APP or HsLON under stringent (preferred), moderately stringent, or non-stringent conditions (see, *e.g.*, *supra*).

β-APP and/or HsLON derivatives may be produced by alteration of their sequences by substitutions, additions or deletions that result in functionally-equivalent molecules. In a specific embodiment of the present invention, the degeneracy of nucleotide coding sequences allows for the use of other DNA sequences that encode substantially the same amino acid sequence as β-APP or HsLON genes. In another specific embodiment, one or more amino acid residues within the sequence of interest may be substituted by another amino acid of a similar polarity and net charge, thus resulting in a silent alteration. Conservative amino acid substitution variants for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. Polar neutral amino acids include glycine, serine, threonine, cysteine,

tyrosine, asparagine, and glutamine. Positively charged (basic) amino acids include arginine, lysine and histidine. Negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

β-APP or HsLON derivatives and analogs of the present invention may be produced by various methodologies known within the art. For example, the cloned β-APP and HsLON gene sequences may be modified by any of numerous methods known within the art. See *e.g.*, Sambrook, *et al.*, 1990. *Molecular Cloning: A Laboratory Manual, 2nd ed.*, (Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY). These sequences may be digested at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification, if so desired, and the resultant fragments isolated and ligated *in vitro*. Additionally, β-APP- or HsLON-encoding nucleic acids may be mutated *in vitro* or *in vivo* to: (*i*) create variations in coding regions; (*ii*) create and/or destroy translation, initiation, and/or termination sequences; and/or (*iii*) form new restriction endonuclease sites or destroy pre-existing ones, so as to facilitate further *in vitro* modification. Any technique for mutagenesis known within the art may be utilized, including but not limited to, chemical mutagenesis and *in vitro* site-directed mutagenesis (see *e.g.*, Hutchinson, *et al.*, 1978. *J. Biol. Chem 253*: 6551-6558); use of TABJ™ linkers (Pharmacia), and other similar methodologies.

Isolation and analysis of the gene product or complex

Once a recombinant cell expressing β-APP and/or HsLON, or a fragment, analog, or derivative thereof, is identified, the individual gene product or complex may be isolated and analyzed. This is achieved by assays that are based upon the physical and/or functional properties of the protein or complex, including, but not limited to, radioactive labeling of the product followed by analysis by gel electrophoresis, immunoassay, cross-linking to marker-labeled products, and the like. The β-APP:HsLON complex may be isolated and purified by standard methods known in the art (either from natural sources or recombinant host cells expressing the proteins/protein complex) including, but not limited to, column chromatography (*e.g.*, ion exchange, affinity, gel exclusion, reverse-phase, high pressure, fast protein liquid, etc), differential centrifugation, differential solubility, or similar methodologies used for the purification of proteins. Alternatively, once β-APP or HsLON or its derivative is identified, the amino acid sequence of the protein can be deduced from the nucleic acid sequence of the chimeric gene from which it was encoded. Hence, the protein or its derivative can be synthesized by standard chemical methodologies known in the art. See, *e.g.*, Hunkapiller, *et al.*, 1984. *Nature* 310: 105-111.

In a specific embodiment, a β-APP:HsLON complex (whether produced by recombinant DNA techniques, chemical synthesis methods, or by purification from native sources) is made up from proteins, fragments, analogs and derivatives thereof, that, as their primary amino acid, contain sequences

substantially as depicted in Figures 1 (SEQ ID NO:2) and 2 (SEQ ID NO:4), as well as proteins homologous thereto.

Manipulations of the β-APP and/or HsLON sequences

Manipulations of the β-APP and/or HsLON sequences may be made at the protein level. Included within the scope of the present invention are complex of the β-APP or HsLON fragments, derivatives, fragments or analogs that are differentially modified during or after translation (*e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, and the like). Any of the numerous chemical modification methodologies known within the art may be utilized including, but not limited to, specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc. In a specific embodiment, the β-APP and/or HsLON sequences are modified to include a fluorescent label. In another specific embodiment, the β-APP and/or the HsLON are modified by the incorporation of a heterofunctional reagent, wherein such heterofunctional reagent may be used to cross-link the members of the complex.

Chemical synthesis

Complexes of analogs and derivatives of β-APP and/or HsLON can be chemically synthesized. For example, a peptide corresponding to a portion of β-APP and/or HsLON that comprises the desired domain or that mediates the desired activity *in vitro* (*e.g.*, β-APP:HsLON complex formation), may be synthesized by use of a peptide synthesizer. In cases where natural products are suspected of being mutant or are isolated from new species, the amino acid sequence of β-APP and/or HsLON isolated from the natural source, as well as those expressed *in vitro*, or from synthesized expression vectors *in vivo* or *in vitro*, may be determined from analysis of the DNA sequence, or alternatively, by direct sequencing of the isolated protein. The β-APP:HsLON complex may also be analyzed by hydrophilicity analysis (see *e.g.*, Hopp & Woods, 1981. *Proc. Natl. Acad. Sci. USA* 78: 3824-3828) that can be utilized to identify the hydrophobic and hydrophilic regions of the proteins, thus aiding in the design of substrates for experimental manipulation, such as in binding experiments, antibody synthesis, etc. Secondary structural analysis may also be performed to identify regions of the β-APP and/or HsLON that assume specific structural motifs. See *e.g.*, Chou & Fasman, 1974. *Biochem.* 13: 222-223. Manipulation, translation, secondary structure prediction, hydrophilicity and hydrophobicity profiles, open reading frame prediction and plotting, and determination of sequence homologies, can be accomplished using computer software programs available in the art, for *e.g.*, Wisconsin GCG (Genetics Computer Group) software package using default parameter settings. Other methods of structural analysis including, but

not limited to, X-ray crystallography (see *e.g.*, Engstrom, 1974. *Biochem. Exp. Biol.* 11: 7-13); mass spectroscopy and gas chromatography (see *e.g.*, METHODS IN PROTEIN SCIENCE, 1997. J. Wiley and Sons, New York, NY) and computer modeling (see *e.g.*, Fletterick & Zoller, eds., 1986. Computer Graphics and Molecular Modeling, In: CURRENT COMMUNICATIONS IN MOLECULAR BIOLOGY, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) may also be employed.

Methodologies for screening

The present invention provides methodologies for screening β-APP, HsLON, and/or β-APP:HsLON complexes, as well as derivatives, fragments and analogs thereof, for the ability to alter and/or modulate cellular functions, particularly those functions in which β-APP and/or HsLON have been implicated. These functions include, but are not limited to, control of cell-cycle progression; regulation of transcription; control of intracellular signal transduction; and pathological processes, as well as various other biological activities (*e.g.*, binding to an anti-β-APP, anti-HsLON, and/or anti-β-APP:HsLON complex antibody, and the like). The derivatives, fragments or analogs that possess the desired immunogenicity and/or antigenicity may be utilized in immunoassays, for immunization, for inhibition of β-APP, HsLON, and/or β-APP:HsLON complex activity, etc. For example, derivatives, fragments or analogs that retain, or alternatively lack or inhibit, a given property of interest (*e.g.*, participation in a β-APP:HsLON complex) may be utilized as inducers, or inhibitors, respectively, of such a property and its physiological correlates. In a specific embodiment, a β-APP:HsLON complex of a fragment of the β-APP and/or a fragment of HsLON that can be bound by an anti-β-APP and/or anti-HsLON antibody or antibody specific for a β-APP:HsLON complex when such a fragment is included within a given β-APP:HsLON complex. Derivatives, fragments and analogs of β-APP:HsLON complex may be analyzed for the desired activity or activities by procedures known within the art.

(2) Production of antibodies to the β-APP:HsLON complex

As disclosed by the present invention herein, β-APP:HsLON complex, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} fragments and an F_{ab} expression library. In a specific embodiment, antibodies to a complex of human β-APP and human HsLON are disclosed. In another specific embodiment, complex formed from fragments of β-APP and HsLON; wherein these fragments contain the protein domain that interacts with the other member of the complex and are used as immunogens for antibody production. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to a β-APP:HsLON complex, or derivative, fragment, analog or homolog thereof.

For the production of polyclonal antibodies, various host animals may be immunized by injection with the native β-APP:HsLON complex, or a synthetic variant thereof, or a derivative of the foregoing (*e.g.*, a cross-linked β-APP:HsLON). Various adjuvants may be used to increase the immunological response and include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.) and human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium parvum*.

For preparation of monoclonal antibodies directed towards a β-APP:HsLON complex, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (see Kohler & Milstein, 1975. *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (see Kozbor, *et al.*, 1983. *Immunol. Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, *et al.*, 1985. In: *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by the use of human hybridomas (see Cote, *et al.*, 1983. *Proc. Natl. Acad. Sci. USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (see Cole, *et al.*, 1985. In: *Monoclonal Antibodies and Cancer Therapy* (Alan R. Liss, Inc., pp. 77-96).

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to β-APP:HsLON complex (see *e.g.*, U.S. Patent No. 4,946,778). In addition, methodologies can be adapted for the construction of F_{ab} expression libraries (see *e.g.*, Huse, *et al.*, 1989. *Science* 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for β-APP:HsLON or derivatives, fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. See *e.g.*, U.S. Patent No. 5,225,539. Antibody fragments that contain the idiotypes to β-APP:HsLON complex may be produced by techniques known in the art including, but not limited to: (*i*) an $F_{(ab')2}$ fragment produced by pepsin digestion of an antibody molecule; (*ii*) an F_{ab} fragment generated by reducing the disulfide bridges of an $F_{(ab')2}$ fragment; (*iii*) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (*iv*) F_v fragments.

In one embodiment, methodologies for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of β-APP:HsLON complex is facilitated by generation of hybridomas that bind to the fragment of β-APP:HsLON complex possessing such a domain. In

another specific embodiment, methodologies for the selection of an antibody that specifically binds a β-APP:HsLON complex but that does not specifically bind to the individual proteins of β-APP:HsLON complex (identified by selecting the antibody on the basis of positive-binding to β-APP:HsLON complex with a concomitant lack of binding to the individual β-APP and HsLON protein) are within the scope of the invention. Accordingly, antibodies that are specific for a domain within β-APP:HsLON complex, or derivative, fragments, analogs or homologs thereof, are also provided herein.

It should be noted that the aforementioned antibodies may be used in methods known within the art relating to the localization and/or quantitation of β-APP:HsLON complex (*e.g.*, for use in measuring levels of the protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, anti-β-APP, anti-HsLON, and/or anti-β-APP:HsLON complex antibodies, or derivatives, fragments, analogs or homologs thereof that contain the antibody derived binding domain, are utilized as pharmacologically-active compounds [hereinafter "Therapeutics"].

(3) Use of β-APP:HsLON Complex in Diagnosis, Prognosis and Screening

β-APP:HsLON complex may serve as a "marker" for specific disease states that involve the disruption of physiological processes in which β-APP and HsLON are known to be involved. See, *e.g.*, BACKGROUND OF THE INVENTION. Such functional activities include, but not limited to, (1) neurodegenerative disorders such as Alzheimer's Disease, dementia of trisomy 21, Parkinson's disease, amyotrophic lateral sclerosis (ALS); (2) cardiomyopathy; (3) some forms of diabetes; (4) hearing loss; (5) and male infertility; as well as (6) a number of less common syndromes and disorders associated with mitochondrial DNA mutations, and the like. Thus β-APP:HsLON complexes are predicted to have diagnostic utility. Therefore, the differentiation and classification of particular groups of patients possessing elevations or deficiencies of a β-APP:HsLON complex may lead to new nosological classifications of diseases, thereby markedly advancing diagnostic ability.

The detection of levels of β-APP:HsLON complex or levels of β-APP and/or HsLON protein, or detection of levels of mRNAs that encode the components of a β-APP:HsLON complex, may be utilized in the analysis of various diseases, and may provide critical information in various medical processes, including: diagnosis, prognosis, identifying disease states, following a disease course, following the efficacy of an administered therapeutics, following therapeutic response, and the like. Similarly, both the nucleic acid sequences (and sequences complementary thereto) and antibodies specific to β-APP:HsLON complex and/or the individual components that can form β-APP:HsLON complexes, can be used in diagnostics.

Said molecules may be utilized in assays (*e.g.*, immunoassays) to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders characterized by aberrant levels of β-APP:HsLON complex, or monitor the treatment thereof. An "aberrant level" means an increased or decreased level in a sample relative to that present in an analogous sample from an unaffected part of the body, or from a subject not having the disorder. The aforementioned immunoassay may be performed by a methodology comprising contacting a sample derived from a patient with an anti-β-APP, anti-HsLON, and/or anti-β-APP:HsLON complex antibody under conditions such that immunospecific-binding may occur, and subsequently detecting or measuring the amount of any immunospecific-binding by the antibody. In a specific embodiment, an antibody specific for β-APP, HsLON, and/or a β-APP:HsLON complex may be used to analyze a tissue or serum sample from a patient for the presence of uncomplexed or complexed β-APP:HsLON; wherein an aberrant level of β-APP, HsLON, and/or β-APP:HsLON complex is indicative of a diseased condition. The immunoassays that may be utilized include, but are not limited to, competitive and non-competitive assay systems using techniques such as Western Blots, radioimmunoassays (RIA), enzyme linked immunosorbent assay (ELISA), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein-A immunoassays, etc.

The nucleic acid species of the present invention encoding the associated protein components of β-APP:HsLON complex, and related nucleotide sequences and subsequences, may also be used in hybridization assays. β-APP and HsLON nucleotide sequences, or subsequences thereof comprising at least 6 nucleotides, may be used as hybridization probes. Hybridization assays can be used to detect, prognose, diagnose, or monitor conditions, disorders, or disease states associated with aberrant levels of the mRNAs encoding the components of a β-APP:HsLON complex, as described *supra*. In specific embodiments of the present invention, diseases and disorders involving or characterized by aberrant levels of β-APP:HsLON complex or a predisposition to develop such disorders may be diagnosed by detecting aberrant levels of β-APP:HsLON complex, or non-complexed β-APP and/or HsLON proteins or nucleic acids for functional activity. This aforementioned functional activity may include, but is not restricted to, (*i*) binding to an interacting partner (*e.g.*, β-APP, HsLON) or (*ii*) detecting mutations in β-APP and/or a HsLON RNA, DNA or protein (*e.g.*, translocations, truncations, changes in nucleotide or amino acid sequence relative to wild-type β-APP and/or the HsLON) that can cause altered expression or activity of a β-APP, a HsLON or a β-APP:HsLON complex.

Methodologies that are well-known within the art (*e.g.*, immunoassays, nucleic acid hybridization assays, biological activity assays, and the like) may be used to determine whether one or more particular β-APP:HsLON complexes are present at either increased or decreased levels, or are

absent, within samples derived from patients suffering from a particular disease or disorder, or possessing a predisposition to develop such a disease or disorder, as compared to the levels in samples from subjects not having such disease or disorder or predisposition thereto. Additionally, these assays may be utilized to determine whether the ratio of β-APP:HsLON complex to the non-complexed components (*i.e.* β-APP and/or HsLON) in the complex of interest is increased or decreased in samples from patients suffering from a particular disease or disorder or having a predisposition to develop such a disease or disorder as compared to the ratio in samples from subjects not having such a disease or disorder or predisposition thereto.

Accordingly, in specific embodiments of the present invention, diseases and disorders that involve altered levels of one or more β-APP:HsLON complex may be diagnosed, or their suspected presence may be screened for, or a predisposition to develop such diseases and disorders may be detected, by quantitatively ascertaining altered levels of: (*i*) the one or more β-APP:HsLON complex; (*ii*) the mRNA encoding both protein members of said complex; (*iii*) the complex functional activity or (*iv*) mutations in β-APP or the HsLON (*e.g.*, translocations in nucleic acids, truncations in the gene or protein, changes in nucleotide or amino acid sequence relative to wild-type β-APP or the HsLON) that enhance/inhibit or stabilize/destabilize β-APP:HsLON complex formation.

In the practice of the present invention, the use of detection techniques, especially those involving antibodies directed against β-APP:HsLON complex, provide methods for the detection of specific cells that express the uncomplexed or complexed protein of interest, *e.g.*, β-APP and/or HsLON. Using such assays, specific cell types may be quantitatively characterized in which one or more particular components of a β-APP:HsLON complex are expressed, and the presence of the uncomplexed or complexed protein may be correlated with cell viability by techniques well-known within the art (*e.g.*, fluorescence-activated cell sorting). Also embodied herein are methodologies directed to the detection of a β-APP:HsLON complex within *in vitro* cell culture models that express a particular β-APP:HsLON complex, or derivatives thereof, for the purpose of characterizing and/or isolating β-APP:HsLON complex. These detection techniques include, but are not limited to, cell-sorting of prokaryotes (see *e.g.*, Davey & Kell, 1996. *Microbiol. Rev.* 60: 641-696); primary cultures and tissue specimens from eukaryotes, including mammalian species such as human (see *e.g.*, Steele, *et al.*, 1996. *Clin. Obstet. Gynecol.* 39: 801-813) and continuous cell cultures (see *e.g.*, Orfao & Ruiz-Arguelles, 1996. *Clin. Biochem.* 29: 5-9.

The present invention additionally provides kits for diagnostic use that are comprised of one or more containers containing an anti-β-APP, anti-HsLON, and/or anti-β-APP:HsLON complex antibody and, optionally, a labeled binding partner to said antibody. The label incorporated into the anti-β-APP:HsLON complex antibody may include, but is not limited to, a chemiluminescent,

enzymatic, fluorescent, colorimetric or radioactive moiety. In another specific embodiment, kits for diagnostic use that are comprised of one or more containers containing modified or unmodified nucleic acids that encode, or alternatively, that are the complement to, β-APP, HsLON, and/or β-APP:HsLON complex and, optionally, a labeled binding partner to said nucleic acids, are also provided. In an alternative specific embodiment, the kit may comprise, in one or more containers, a pair of oligonucleotide primers (*e.g.*, each 6-30 nucleotides in length) that are capable of acting as amplification primers for polymerase chain reaction (PCR; see *e.g.*, Innis, *et al.*, 1990. PCR PROTOCOLS, Academic Press, Inc., San Diego, CA), ligase chain reaction, cyclic probe reaction, and the like, or other methods known within the art. The kit may, optionally, further comprise a predetermined amount of a purified β-APP, HsLON or β-APP:HsLON complex, or nucleic acids thereof, for use as a diagnostic, standard, or control in the aforementioned assays.

(4) Therapeutic uses of β-APP and HsLON proteins and β-APP:HsLON complexes

The present invention provides a method for treatment or prevention of various diseases and disorders by administration of a biologically-active therapeutic compound (hereinafter "Therapeutic"). Such "Therapeutics" include but are not limited to: (i) β-APP, HsLON, and β-APP:HsLON complex, and derivative, fragments, analogs and homologs thereof; (ii) antibodies directed against the aforementioned proteins and protein complex thereof; (iii) nucleic acids encoding β-APP and/or HsLON, and derivatives, fragments, analogs and homologs thereof; (iv) antisense nucleic acids to sequences encoding β-APP and HsLON proteins, and (v) β-APP:HsLON complex and modulators thereof (*i.e.*, inhibitors, agonists and antagonists).

As previously discussed, β-APP and its binding partner HsLON, are implicated significantly in disorders including, but not limited to, neurodegenerative disorders such as Alzheimer's Disease, dementia of trisomy 21, Parkinson's disease, amyotrophic lateral sclerosis (ALS); cardiomyopathy; some forms of diabetes; hearing loss; and male infertility; as well as a number of less common syndromes and disorders associated with mitochondrial DNA mutations. A wide range of cell diseases is treated or prevented by administration of a Therapeutic that modulates, *i.e.*, antagonizes or promotes, β-APP:HsLON complex activity or formation.

Diseases or disorders associated with aberrant levels of a β-APP:HsLON complex or levels of activity or aberrant levels of β-APP may be treated by administration of a Therapeutic that modulates β-APP:HsLON complex formation or activity. In a specific embodiment, the activity or levels of β-APP are modulated by administration of HsLON. In another specific embodiment, the activity or levels of HsLON are modulated by administration of β-APP.

Disorders with Increased β-APP and β-APP:HsLON Complex Levels

Diseases and disorders that are characterized by increased (relative to a subject not suffering from said disease or disorder) β-APP:HsLON levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) β-APP:HsLON complex formation or activity. Therapeutics that antagonize β-APP:HsLON complex formation or activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, β-APP or HsLON, or analogs, derivatives, fragments or homologs thereof; (*ii*) anti-β-APP, anti-HsLON, and/or anti-β-APP:HsLON complex antibodies; (*iii*) nucleic acids encoding β-APP or HsLON; (*iv*) concurrent administration of a β-APP and a HsLON antisense nucleic acid and β-APP and/or HsLON nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous [non-β-APP and/or non-HsLON] insertion within the coding sequences of β-APP and HsLON coding sequences) are utilized to "knockout" endogenous β-APP and/or HsLON function by homologous recombination (see *e.g.*, Capecchi, 1989. *Science* 244: 1288-1292). In an additional embodiment of the present invention, mutants or derivatives of a first HsLON that possess greater affinity for β-APP than the wild-type first HsLON may be administered to compete with a second HsLON for binding to β-APP, thereby reducing the levels of complex between β-APP and the second HsLON.

Increased levels of β-APP:HsLON complex can be readily detected by quantifying protein and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or protein levels, structure and/or activity of the expressed β-APP:HsLON complex (or β-APP and HsLON mRNAs). Methods that are well-known within the art include, but are not limited to, immunoassays to detect β-APP:HsLON complex (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, and the like) and/or hybridization assays to detect concurrent expression of β-APP and HsLON mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, and the like).

Reduction of β-APP and β-APP:HsLON Complex Expression

A specific embodiment of the present invention discloses methods for the reduction of β-APP:HsLON complex expression (*i.e.*, the expression of the two protein components of the complex and/or formation of the complex) by targeting mRNAs that express the protein moieties. RNA Therapeutics are differentiated into three classes: (*i*) antisense species; (*ii*) ribozymes or (*iii*) RNA aptamers. See *e.g.*, Good, *et al.*, 1997. *Gene Therapy* 4: 45-54. Antisense oligonucleotides have been the most widely utilized and are discussed *infra*. Ribozyme therapy involves the administration (*i.e.*, induced expression) of small RNA molecules with enzymatic ability to cleave, bind, or otherwise inactivate specific RNAs, thus reducing or eliminating the expression of particular proteins. See *e.g.*,

Grassi & Marini, 1996. *Ann. Med.* 28: 499-510. At present, the design of "hairpin" and/or "hammerhead" RNA ribozymes are necessary to specifically-target a particular mRNA (*e.g.*, β-APP mRNA). RNA aptamers are specific RNA ligands for proteins, such as for *Tat* and *Rev* RNA (see *e.g.*, Good, *et al.*, 1997. *Gene Therapy* 4: 45-54) which can specifically inhibit their translation.

In a preferred embodiment of the present invention, the activity or level of β-APP may be reduced by administration of HsLON, a nucleic acid that encodes HsLON or an antibody (or a derivative or fragment of the antibody possessing the binding domain thereof) that immunospecifically-binds to HsLON. Similarly, the levels or activity of HsLON may be reduced by administration of β-APP, a nucleic acid encoding β-APP or an antibody (or a derivative or fragment of the antibody possessing the binding domain thereof) that immunospecifically-binds β-APP. In another embodiment of the present invention, diseases or disorders that are associated with increased levels of β-APP or HsLON, may be treated or prevented by administration of a Therapeutic that increases β-APP:HsLON complex formation, if said complex formation acts to reduce or inactivate β-APP or the particular HsLON via β-APP:HsLON complex formation. Such diseases or disorders may be treated or prevented by: (*i*) the administration of one member of β-APP:HsLON complex, including mutants of one or both of the proteins that possess increased affinity for the other member of β-APP:HsLON complex (so as to cause increased complex formation) or (*ii*) the administration of antibodies or other molecules that serve to stabilize β-APP:HsLON complex, or the like.

(5) Determination of the Biological Effect of the Therapeutic

In preferred embodiments of the present invention, suitable *in vitro* or *in vivo* assays are utilized to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts the desired effect upon said cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

Neurodegenerative disorders

β-APP and its binding partner HsLON have been implicated in neurodegenerative diseases. Accordingly, Therapeutics of the invention, particularly, but not limited to, those that modulate (or

supply) β-APP:HsLON complex activity, may be effective in treating or preventing neurodegenerative disease. Therapeutics of the present invention that modulate β-APP:HsLON complex activity involved in neurodegenerative disorders can be assayed by any method known in the art for efficacy in treating or preventing such neurodegenerative diseases and disorders. Such assays include *in vitro* assays for regulated cell maturation or inhibition of apoptosis or *in vivo* assays using animal models of neurodegenerative diseases or disorders, or any of the assays described *infra*. Potentially effective Therapeutics, for example but not by way of limitation, promote regulated cell maturation and prevent cell apoptosis in culture, or reduce neurodegeneration in animal models in comparison to controls.

Once a neurodegenerative disease or disorder has been shown to be amenable to treatment by modulation of β-APP:HsLON complex activity, that neurodegenerative disease or disorder can be treated or prevented by administration of a Therapeutic that modulates β-APP:HsLON complex formation or activity, including supplying a β-APP:HsLON complex or an uncomplexed binding partner, *e.g.*, β-APP and/or HsLON. Such diseases include all degenerative disorders involved with aging, especially osteoarthritis and neurodegenerative disorders.

Hypertrophic cardiomyopathy

HsLON has been linked to several disorders and syndromes that share the common feature of hypertrophic cardiomyopathy. Accordingly, Therapeutics of the invention, particularly those that modulate (or supply) β-APP:HsLON complex activity or formation may be effective in treating or preventing hypertrophic cardiomyopathy and associated diseases or disorders. Therapeutics of the invention (particularly Therapeutics that modulate the levels or activity of a β-APP:HsLON complex) can be assayed by any method known in the art, including those described *infra*, for efficacy in treating or preventing such diseases and disorders.

Accordingly, once an hypertrophic cardiomyopathy-associated disease or disorder has been shown to be amenable to treatment by modulation of β-APP:HsLON complex activity or formation, that disease or disorder can be treated or prevented by administration of a Therapeutic that modulates β-APP:HsLON complex activity or formation including supplying a β-APP:HsLON complex, or individual uncomplexed β-APP and/or HsLON proteins.

Diabetes

HsLON has been implicated in the development of maternally-inherited diabetes. Accordingly, Therapeutics of the invention, particularly, but not limited to, those that modulate (or alter or supply) β-APP:HsLON complex activity, may be effective in treating or preventing neurodegenerative disease.

Therapeutics of the present invention that modulate β-APP:HsLON complex activity involved in diabetes can be assayed by any method known in the art for efficacy in treating or preventing such diseases and disorders. Such assays include *in vitro* assays or *in vivo* assays using animal models of diabetes, or any of the assays described *infra*. Potentially effective Therapeutics, for example but not by way of limitation, reduce diabetes in animal models in comparison to controls.

Once a diabetes has been shown to be amenable to treatment by modulation of β-APP:HsLON complex activity, that disease or disorder can be treated or prevented by administration of a Therapeutic that modulates β-APP:HsLON complex formation or activity, including supplying a β-APP:HsLON complex or an uncomplexed binding partner, *e.g.*, β-APP and/or HsLON.

Hearing Loss

HsLON has been implicated in both sensorineural hearing loss and maternally-inherited deafness. Accordingly, Therapeutics of the invention, particularly but not limited to those that modulate (or supply) β-APP:HsLON complex activity, may be effective in treating or preventing sensorineural hearing loss or maternally-inherited deafness. Therapeutics of the present invention that modulate β-APP:HsLON complex activity involved in sensorineural hearing loss or maternally-inherited deafness can be assayed by any method known in the art for efficacy in treating or preventing such diseases and disorders. Such assays include *in vitro* assays or *in vivo* assays using animal models of sensorineural hearing loss or maternally-inherited deafness, or any of the assays described *infra*. Potentially effective Therapeutics, for example but not by way of limitation, reduce sensorineural hearing loss or maternally-inherited deafness in animal models in comparison to controls.

Once a sensorineural hearing loss or maternally-inherited deafness has been shown to be amenable to treatment by modulation of β-APP:HsLON complex activity, that disease or disorder can be treated or prevented by administration of a Therapeutic that modulates β-APP:HsLON complex formation or activity, including supplying a β-APP:HsLON complex or an uncomplexed binding partner, *e.g.*, β-APP and/or HsLON.

Male Infertility

HsLON has been implicated in the development of male infertility. Accordingly, Therapeutics of the invention, particularly but not limited to those that modulate (or supply) β-APP:HsLON complex activity, may be effective in treating or preventing male infertility. Therapeutics of the present invention that modulate β-APP:HsLON complex activity involved in male infertility can be assayed by any method known in the art for efficacy in treating or preventing such diseases and disorders. Such assays

include *in vitro* assays or *in vivo* assays using animal models of male infertility, or any of the assays described *infra*. Potentially effective Therapeutics, for example but not by way of limitation, reduce male infertility in animal models in comparison to controls.

Once a male infertility has been shown to be amenable to treatment by modulation of β-APP:HsLON complex activity, that disease or disorder can be treated or prevented by administration of a Therapeutic that modulates β-APP:HsLON complex formation or activity, including supplying a β-APP:HsLON complex or an uncomplexed binding partner, *e.g.*, β-APP and/or HsLON.

Diseases associated with mitochondrial DNA mutations

HsLON has been implicated in a number of diseases or disorders associated with mitochondrial DNA mutations. Accordingly, Therapeutics of the invention, particularly but not limited to those that modulate (or supply) β-APP:HsLON complex activity, may be effective in treating or preventing diseases or disorders associated with mitochondrial DNA mutations. Therapeutics of the present invention that modulate β-APP:HsLON complex activity involved in diseases or disorders associated with mitochondrial DNA mutations can be assayed by any method known in the art for efficacy in treating or preventing such diseases and disorders. Such assays include *in vitro* assays or *in vivo* assays using animal models of diseases or disorders associated with mitochondrial DNA mutations, or any of the assays described *infra*. Potentially effective Therapeutics, for example but not by way of limitation, reduce diseases or disorders associated with mitochondrial DNA mutations in animal models in comparison to controls.

Once diseases or disorders associated with mitochondrial DNA mutations have been shown to be amenable to treatment by modulation of β-APP:HsLON complex activity, that disease or disorder can be treated or prevented by administration of a Therapeutic that modulates β-APP:HsLON complex formation or activity, including supplying a β-APP:HsLON complex or an uncomplexed binding partner, *e.g.*, β-APP and/or HsLON.

(6) Gene Therapy

In a specific embodiment of the present invention, nucleic acids comprising a sequence that encodes β-APP and/or HsLON, or functional derivatives thereof, are administered to modulate β-APP:HsLON complex function, by way of gene therapy. In more specific embodiments, a nucleic acid or nucleic acids encoding both β-APP and HsLON, or functional derivatives thereof, are administered by way of gene therapy. Gene therapy refers to therapy that is performed by the administration of a specific nucleic acid to a subject. In this embodiment of the present invention, the nucleic acid produces its

encoded protein(s), which then serve to exert a therapeutic effect by modulating β-APP:HsLON complex function. Any of the methodologies relating to gene therapy available within the art may be used in the practice of the present invention. See *e.g.*, Goldspiel, *et al.*, 1993. *Clin. Pharm.* 12: 488-505.

In a preferred embodiment, the Therapeutic comprises a β-APP and/or HsLON nucleic acid that is part of an expression vector expressing both of the aforementioned proteins, or fragments or chimeric proteins thereof, within a suitable host. In a specific embodiment, such a nucleic acid possesses a promoter that is operably-linked to β-APP and HsLON coding region(s), or, less preferably, two separate promoters linked to separate β-APP and HsLON coding regions. Said promoter may be inducible or constitutive, and, optionally, tissue-specific. In another specific embodiment, a nucleic acid molecule is used in which β-APP and HsLON coding sequences (and any other desired sequences) are flanked by regions that promote homologous recombination at a desired site within the genome, thus providing for intra-chromosomal expression of β-APP and HsLON nucleic acids. See *e.g.*, Koller & Smithies, 1989. *Proc. Natl. Acad. Sci. USA* 86: 8932-8935.

Delivery of the Therapeutic nucleic acid into a patient may be either direct (*i.e.*, the patient is directly exposed to the nucleic acid or nucleic acid-containing vector) or indirect (*i.e.*, cells are first transformed with the nucleic acid *in vitro*, then transplanted into the patient). These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy. In a specific embodiment of the present invention, a nucleic acid is directly administered *in vivo*, where it is expressed to produce the encoded product. This may be accomplished by any of numerous methods known in the art including, but not limited to, constructing said nucleic acid as part of an appropriate nucleic acid expression vector and administering the same in a manner such that it becomes intracellular (*e.g.*, by infection using a defective or attenuated retroviral or other viral vector; see U.S. Patent No. 4,980,286); directly injecting naked DNA; using microparticle bombardment (*e.g.*, a "Gene Gun®; Biolistic, DuPont); coating said nucleic acids with lipids; using associated cell-surface receptors/transfecting agents; encapsulating in liposomes, microparticles, or microcapsules; administering it in linkage to a peptide that is known to enter the nucleus; or by administering it in linkage to a ligand predisposed to receptor-mediated endocytosis (see *e.g.*, Wu & Wu, 1987. *J. Biol. Chem.* 262: 4429-4432), which can be used to "target" cell types that specifically express the receptors of interest, etc.

In another specific embodiment of the present invention, a nucleic acid-ligand complex may be produced in which the ligand comprises a fusogenic viral peptide designed so as to disrupt endosomes, thus allowing the nucleic acid to avoid subsequent lysosomal degradation. In yet another specific embodiment, the nucleic acid may be targeted *in vivo* for cell-specific endocytosis and expression, by targeting a specific receptor. See *e.g.*, PCT Publications WO 92/06180; WO93/14188 and WO 93/20221. Alternatively, the nucleic acid may be introduced intracellularly and incorporated within

a host cell genome for expression by homologous recombination. See *e.g.*, Zijlstra, *et al.*, 1989. *Nature* 342: 435-438.

In yet another specific embodiment, a viral vector that contains β-APP and/or HsLON nucleic acids is utilized. For example, retroviral vectors may be employed (see *e.g.*, Miller, *et al.*, 1993. *Meth. Enzymol.* 217: 581-599) that have been modified to delete those retroviral-specific sequences that are not required for packaging of the viral genome, with its subsequent integration into host cell DNA. β-APP and/or HsLON (preferably both) nucleic acids may be cloned into a vector that facilitates delivery of the genes into a patient. See *e.g.*, Boesen, *et al.*, 1994. *Biotherapy* 6: 291-302; Kiem, *et al.*, 1994. *Blood* 83: 1467-1473. Additionally, adenovirus may be used as an especially efficacious "vehicle" for the delivery of genes to the respiratory epithelia. Other targets for adenovirus-based delivery systems are liver, central nervous system, endothelial cells, and muscle. Adenoviruses also possess advantageous abilities to infect non-dividing cells. For a review see *e.g.*, Kozarsky & Wilson, 1993. *Curr. Opin. Gen. Develop.* 3: 499-503. Adenovirus-associated virus (AAV) has also been proposed for use in gene therapy. See *e.g.*, Walsh, *et al.*, 1993. *Proc. Soc. Exp. Biol. Med.* 204: 289-300.

An additional approach to gene therapy in the practice of the present invention involves transferring a gene into cells in *in vitro* tissue culture by such methods as electroporation, lipofection, calcium phosphate-mediated transfection, viral infection, or the like. Generally, the methodology of transfer includes the concomitant transfer of a selectable marker to the cells. The cells are then placed under selection pressure (*e.g.*, antibiotic resistance) so as to facilitate the isolation of those cells that have taken up, and are expressing, the transferred gene. Those cells are then delivered to a patient. In a specific embodiment, prior to the *in vivo* administration of the resulting recombinant cell, the nucleic acid is introduced into a cell by any method known within the art including, but not limited to: transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences of interest, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, and similar methodologies that ensure that the necessary developmental and physiological functions of the recipient cells are not disrupted by the transfer. See *e.g.*, Loeffler & Behr, 1993. *Meth. Enzymol.* 217: 599-618. The chosen technique should provide for the stable transfer of the nucleic acid to the cell, such that the nucleic acid is expressible by the cell. Preferably, said transferred nucleic acid is heritable and expressible by the cell progeny.

In preferred embodiments of the present invention, the resulting recombinant cells may be delivered to a patient by various methods known within the art including, but not limited to, injection of epithelial cells (*e.g.*, subcutaneously), application of recombinant skin cells as a skin graft onto the patient, and intravenous injection of recombinant blood cells (*e.g.*, hematopoietic stem or progenitor

cells). The total amount of cells that are envisioned for use depend upon the desired effect, patient state, and the like, and may be determined by one skilled within the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and may be xenogeneic, heterogeneic, syngeneic, or autogeneic. Cell types include, but are not limited to, differentiated cells such as epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes and blood cells, or various stem or progenitor cells, in particular embryonic heart muscle cells, liver stem cells (International Patent Publication WO 94/08598), neural stem cells (Stemple and Anderson, 1992, *Cell 71*: 973-985), hematopoietic stem or progenitor cells, *e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, and the like. In a preferred embodiment, the cells utilized for gene therapy are autologous to the patient.

In a specific embodiment in which recombinant cells are used in gene therapy, stem or progenitor cells that can be isolated and maintained *in vitro* may be utilized. Such stem cells include, but are not limited to, hematopoietic stem cells (HSC), stem cells of epithelial tissues, and neural stem cells (see *e.g.*, Stemple & Anderson, 1992. *Cell* 71: 973-985). With respect to HSCs, any technique that provides for the isolation, propagation, and maintenance *in vitro* of HSC may be used in this specific embodiment of the invention. As previously discussed, the HSCs utilized for gene therapy may, preferably, be autologous to the patient. When used, non-autologous HSCs are, preferably, utilized in conjunction with a method of suppressing transplantation immune reactions of the future host/patient. See *e.g.*, Kodo, *et al.*, 1984. *J. Clin. Invest.* 73: 1377-1384. In another preferred embodiment of the present invention, HSCs may be highly enriched (or produced in a substantially-pure form), by any techniques known within the art, prior to administration to the patient. See *e.g.*, Witlock & Witte, 1982. *Proc. Natl. Acad. Sci. USA* 79: 3608-3612.

(7) Utilization of Anti-Sense Oligonucleotides

In a specific embodiment of the present invention, β-APP, HsLON, and/or β-APP:HsLON complex formation and function may be inhibited by the use of anti-sense nucleic acids for β-APP or HsLON, or most preferably, β-APP and HsLON. In addition, the present invention discloses the therapeutic or prophylactic use of nucleic acids (of at least six nucleotides in length) that are anti-sense to a genomic sequence (gene) or cDNA encoding β-APP and/or HsLON, or portions thereof. Such anti-sense nucleic acids have utility as Therapeutics that inhibit β-APP, HsLON, and/or β-APP:HsLON complex formation or activity, and may be utilized in a therapeutic or prophylactic manner.

Another specific embodiment of the present invention discloses methodologies for inhibition of expression of β-APP and HsLON nucleic acid sequences within a prokaryotic or eukaryotic cell, such as

providing a cell with an therapeutically-effective amount of an anti-sense nucleic acid of β-APP and/or HsLON, or derivatives thereof.

The anti-sense nucleic acids of the present invention may be oligonucleotides that may either be directly administered to a cell or that may be produced *in vivo* by transcription of the exogenous, introduced sequences. In addition, the anti-sense nucleic acid may be complementary to either a coding (*i.e.*, exonic) and/or non-coding (*i.e.*, intronic) region of β-APP or HsLON mRNAs. β-APP and HsLON anti-sense nucleic acids are, at least, six nucleotides in length and are, preferably, oligonucleotides ranging from 6-200 nucleotides in length. In specific embodiments, the anti-sense oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The anti-sense oligonucleotides may be DNA or RNA (or chimeric mixtures, derivatives or modified versions thereof), may be either single-stranded or double-stranded and may be modified at a base, sugar or phosphate backbone moiety.

In addition, said anti-sense oligonucleotide may include other associated functional groups, such as peptides, moieties that facilitate the transport of the oligonucleotide across the cell membrane, hybridization-triggered cross-linking agents, hybridization-triggered cleavage-agents, and the like. See *e.g.*, Letsinger, *et al.*, 1989. *Proc. Natl. Acad. Sci. U.S.A.* 86: 6553-6556; PCT Publication No. WO 88/09810. In a specific embodiment, β-APP and HsLON antisense oligonucleotides comprise catalytic RNAs or ribozymes. See, *e.g.*, Sarver, *et al.*, 1990. *Science* 247: 1222-1225.

The anti-sense oligonucleotides of the present invention may be synthesized by standard methodologies known within the art including, but not limited to: (*i*) automated phosphorothioate-mediated oligonucleotide synthesis (see *e.g.*, Stein, *et al.*, 1988. *Nuc. Acids Res.* 16: 3209) or (*ii*) methylphosphonate oligonucleotides prepared by use of controlled pore glass polymer supports (see *e.g.*, Sarin, *et al.*, 1988. *Proc. Natl. Acad. Sci. U.S.A.* 85: 7448-7451).

In an alternative embodiment, β-APP and HsLON antisense nucleic acids are produced intracellularly by transcription of an exogenous sequence. For example, a vector comprising a promoter functionally linked to the reverse complement of a desired gene, and the like, may be produced that (upon being taken up by the cell) is transcribed *in vivo*, thus producing an antisense nucleic acid (RNA) species. The aforementioned vector may either remain episomal or become chromosomally-integrated, so long as it can be transcribed to produce the desired antisense RNA. An origin of the vectors utilized may be derived from bacterial, viral, yeast or other sources known within the art that are utilized for replication and expression in mammalian cells. Expression of the sequences encoding β-APP and HsLON antisense RNAs may be facilitated by any promoter known within the art to function in mammalian, preferably, human cells. Such promoters may be inducible or constitutive and include, but

are not limited to: (*i*) the SV40 early promoter region; (*ii*) the promoter contained in the 3'-terminus long terminal repeat of Rous sarcoma virus (RSV); (*iii*) the Herpesvirus thymidine kinase promoter and (*iv*) the regulatory sequences of the metallothionein gene.

β-APP and HsLON antisense nucleic acids may be utilized prophylactically or therapeutically in the treatment or prevention of disorders of a cell type that expresses (or preferably over-expresses) β-APP, HsLON, and/or β-APP:HsLON complex. Cell types that express or over-express β-APP and HsLON RNA may be identified by various methods known within the art including, but not limited to, hybridization with β-APP and HsLON-specific nucleic acids (*e.g.*, by Northern hybridization, dot blot hybridization, *in situ* hybridization) or by observing the ability of RNA from the specific cell type to be translated *in vitro* into β-APP and/or HsLON by immunohistochemistry. In a preferred aspect, primary tissue from a patient may be assayed for β-APP and/or HsLON expression by, for example, immunocytochemistry or *in situ* hybridization, prior to actual treatment.

Pharmaceutical compositions of the present invention, comprising an effective amount of a β-APP and HsLON antisense nucleic acid contained within a pharmaceutically-acceptable carrier, may be administered to a patient having a disease or disorder of a type that involves modified expression of β-APP:HsLON complex, or of RNA or protein of the individual components of said complex. The amount of β-APP and/or HsLON antisense nucleic acid that is effective in the treatment of a particular disorder or condition will be dependant upon the nature of the disorder or condition, and may be determined by standard clinical techniques. Where possible, it is desirable to determine the antisense cytotoxicity in *in vitro* systems and in useful animal model prior to testing and use in humans. In a specific embodiment, pharmaceutical compositions comprising β-APP and HsLON antisense nucleic acids may be administered via liposomes, microparticles, or microcapsules or the like. See, *e.g.*, *supra*, and Leonetti, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 2448-2451.

(8) β-APP:HsLON Complex Assays

The functional activity of β-APP:HsLON complex (and derivatives, fragments, analogs and homologs thereof) may be assayed by a number of methods known in the art. For example, putative modulators (*e.g.*, inhibitors, agonists and antagonists) of β-APP:β-APP complex activity (*e.g.*, anti-β-APP:HsLON complex antibodies, as well as β-APP or HsLON antisense nucleic acids) may be assayed for their ability to modulate β-APP:HsLON complex formation and/or activity.

Immunoassays

In a specific embodiment, immunoassay-based methodologies are provided wherein one is assaying for: (*i*) the ability to bind to, or compete with, wild-type β-APP:HsLON complex or HsLON, or (*ii*) the ability to bind to an anti-β-APP:HsLON complex antibody. These immunoassays include, but are not limited to, competitive and non-competitive assay systems utilizing techniques such as radioimmunoassays, enzyme linked immunosorbent assay (ELISA), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (*e.g.*, using colloidal gold, enzyme or radioisotope labels), complement fixation assays, Western blots, Northwestern blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), immunofluorescence assays, protein-A assays and immunoelectrophoresis assays, and the like. In one specific embodiment, antibody binding is detected directly by assaying for a label on a primary antibody. In another specific embodiment, binding of the primary antibody is ascertained by detection of a secondary antibody (or reagent) that is specific for the primary antibody. In a further embodiment, the secondary antibody is labeled.

Gene Expression Assays

Expression of β-APP or HsLON genes (from both endogenous genes and from incorporated recombinant DNA) may be detected using techniques known within the art including, but not limited to, Southern hybridization, Northern hybridization, restriction endonuclease mapping, DNA sequence analysis, and polymerase chain reaction amplification (PCR) followed by Southern hybridization or RNase protection (see *e.g.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY 1997, John Wiley and Sons, New York, NY) with probes specific for β-APP and HsLON genes in various cell types.

In one specific embodiment of the present invention, Southern hybridization may be used to detect genetic linkage of β-APP and/or HsLON gene mutations to physiological or pathological states. Numerous cell types, at various stages of development, may be characterized for their expression of β-APP and HsLON (particularly the concomitant expression of β-APP and HsLON within the same cells). The stringency of the hybridization conditions for Northern or Southern blot analysis may be manipulated to ensure detection of nucleic acids with the desired degree of relatedness to the specific probes used. See, *e.g.*, *supra*. Modification of these aforementioned methods, as well as other methods well-known within the art, may be utilized in the practice of the present invention.

Binding Assays

Derivatives, fragments, analogs and homologs of HsLON may be assayed for binding to β-APP by any method known within the art including, but not limited to: (*i*) the modified yeast two hybrid assay system; (*ii*) immunoprecipitation with an antibody that binds to β-APP within a complex, followed by analysis by size fractionation of the immunoprecipitated proteins (*e.g.*, by denaturing or non-denaturing polyacrylamide gel electrophoresis); (*iii*) Western analysis; (*v*) non-denaturing gel electrophoresis, and the like. Alternatively, the aforementioned techniques may be modified to allow for the reverse analysis, whereby β-APP components bind to HsLON.

Assays for Biological Activity

A specific embodiment of the present invention provides a methodology for screening a derivative, fragment, analog or homolog of β-APP for biological activity, which is comprised of contacting said derivative, fragment, analog or homolog of β-APP with HsLON and detecting complex formation between said derivative, fragment, analog or homolog of β-APP and HsLON; wherein the detection of the formation of said complex indicates that said β-APP derivative, fragment, analog or homolog, possesses biological (*e.g.*, binding) activity. Similarly, an additional embodiment discloses a methodology for the screening a derivative, fragment, analog or homolog of HsLON for biological activity comprising contacting said derivative, fragment, analog or homolog of said protein with β-APP; and detecting complex formation between said derivative, fragment, analog or homolog of HsLON and β-APP; wherein detecting the formation of said complex indicates that said HsLON derivative, fragment, analog, or homolog possesses biological activity.

Related Treatment Assays

Neurodegeneration

Similarly, once a neurodegeneration disease or disorder has been shown to be amenable to treatment by modulation of β-APP, HsLON, and/or β-APP:HsLON complex activity or formation, that disease or disorder can be treated or prevented by administration of a Therapeutic that modulates β-APP:HsLON complex activity or formation, including supplying β-APP, HsLON, and/or β-APP:HsLON complex. In a specific embodiment, β-APP, HsLON, and/or β-APP:HsLON complex is administered to treat or prevent a neurodegenerative disease or disorder. β-APP has been implicated in the development and involution of all organs, including the central nervous system. Casaccia-Bonnefil *et al.*, 1997, Genes and Dev. 11: 2335-2346. Accordingly, a β-APP:HsLON complex or derivative, homolog, analog or fragment thereof, nucleic acid molecules encoding β-APP or HsLON,

anti-β-APP:HsLON complex antibodies, and other modulators of β-APP, HsLON, and/or β-APP:HsLON complex activity or formation can be tested for activity in treating or preventing neurodegenerative disease in *in vitro* and *in vivo* assays.

In one embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing neurodegenerative disease by contacting a cultured cell that exhibits an indicator of a neurodegenerative disease *in vitro* with the Therapeutic and comparing the level of said indicator in the cell so contacted with the Therapeutic, with said level of said indicator in a cell not so contacted, wherein a lower level in said contacted cell indicates that the Therapeutic has activity in treating or preventing neurodegenerative disease. Specific examples of such cultured models for neurodegenerative disease include, but are not limited to, cultured rat endothelial cells from affected and nonaffected individuals (Maneiro *et al.*, 1997, Methods Find. Exp. Clin. Pharmacol. 19: 5-12); P19 murine embryonal carcinoma cells (Hung *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89: 9439-9443); and dissociated cell cultures of cholinergic neurons from nucleus basalis of Meynert (Nakajima *et al.*, 1985, Proc. Natl. Acad. Sci. USA 82: 6325-6329).

In another embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing neurodegenerative disease by administering the Therapeutic to a test animal that is predisposed to develop symptoms of a neurodegenerative disease, and measuring the change in said symptoms after administration of said Therapeutic, wherein a reduction in the severity of the symptoms of the neurodegenerative disease, or the prevention of the symptoms of the neurodegenerative disease, indicates that the Therapeutic has activity in treating or preventing said disease states. Such a test animal can be any one of a number of animal models known in the art for neurodegenerative disease. These models, including those for Alzheimer's Disease and mental retardation of trisomy 21, accurately mimic natural human neurodegenerative diseases. Farine, 1997, *Toxicol.* 119: 29-35. Examples of specific models include, but are not limited to, the partial trisomy 16 mouse (Holtzman *et al.*, 1996, Proc. *Natl. Acad. Sci. USA* 93: 13333-13338); bilateral nucleus basalis magnocellularis-lesioned rats (Popovic *et al.*, 1996, *Int. J. Neurosci.* 86: 281-299); the aged rat (Muir, 1997, *Pharmacol. Biochem. Behav.* 56: 687-696); the PDAPP transgenic mouse model of Alzheimer's disease (Johnson-Wood *et al.*, 1997, *Proc. Natl. Acad. Sci. USA 94*: 1550-1555); and experimental autoimmune dementia (Oron *et al.*, 1997, *J. Neural Transm.* Suppl. 49: 77-84).

Cardiomyopathy

In a specific embodiment of the present invention, a β-APP:HsLON complex is administered to treat or prevent a disease or disorder involving cardiomyopathy. HsLON has been linked to several disorders and syndromes that share the common feature of hypertrophic cardiomyopathy. Accordingly,

a β-APP:HsLON complex or a derivative, homolog, analog or fragment thereof, nucleic acids encoding the β-APP and HsLON proteins, anti-β-APP:HsLON antibodies, and other modulators of β-APP:HsLON complex activity or formation, can be tested for activity in treating or preventing a disease or disorder involving cardiomyopathy in *in vitro* and *in vivo* assays.

In one embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing cardiomyopathy by contacting cultured cells, such as but not limited to, cultured cardiac myocytes from neonates (Wall *et al.*, 1996, Eur. J. Pharmacol. 306:165-174) or adults (Hain and Schaper, 1996, Curr. Opin. Cardiol. 11:293-301), and cultured autoreactive T cells in autoimmune myocarditis (Perez-Leiros *et al.*, 1997, Neuroimmunomodulation 4:91-97) that exhibit an indicator of cardiomyopathy *in vitro*, with the Therapeutic, and comparing the level of said indicator in the cells contacted with the Therapeutic, with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the Therapeutic has activity in treating or preventing cardiomyopathy.

In another embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing cardiomyopathy by administering the Therapeutic to a test animal exhibiting symptoms of cardiomyopathy; and measuring the change in said symptoms of cardiomyopathy after administration of said Therapeutic, wherein a reduction in the severity of the symptoms, or prevention of the symptoms, of cardiomyopathy indicates that the Therapeutic has activity in treating or preventing cardiomyopathy. Such a test animal can be any one of a number of animal models known in the art for cardiomyopathy. These models include, but are not limited to, those of aryl-hydrocarbon receptor-deficient mice (Fernandez-Salguero *et al.*, 1997, *Vet. Pathol. 34*: 605-614), experimental autoimmune myocarditis in mice (Perez-Leiros *et al.*, 1997, *Neuroimmunomodulation 4*: 91-97), transgenic mice overexpressing tropomodulin (Sussman *et al.*, 1998, *J. Clin. Invest. 101:* 51-61), guinea pigs immunized with adenine nucleotide transporter type I (Dorner *et al.*, 1997, *Mol. Cell. Biochem. 174*: 261-269), MLP-deficient mice (Arber *et al.*, 1997, *Cell 88*: 393-403), and adenine nucleotide translocator type I – knockout mice (Graham *et al.*, 1997, *Nat. Genet. 16*: 226-234). The relationship of cardiomyopathy in these models to heart disease in general is well reviewed by Christensen *et al.*, 1997, *Am. J. Physiol.* 272: H2513-H2524.

Diabetes

In yet another specific embodiment of the present invention, a β-APP:HsLON complex is administered to a subject to treat or prevent diabetes in the subject. HsLON has been implicated in the development of maternally-inherited diabetes. Accordingly, a β-APP:HsLON complex or a derivative, homolog, analog or fragment thereof, nucleic acids encoding the β-APP and HsLON proteins, anti-β-APP:HsLON antibodies, and other modulators of β-APP:HsLON complex activity or formation,

can be tested for activity in treating or preventing diabetes in *in vitro* and *in vivo* assays.

In one embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing diabetes by contacting cultured cells that exhibit an indicator of diabetes *in vitro* with the Therapeutic, and comparing the level of said indicator in the cells contacted with the Therapeutic, with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the Therapeutic has activity in treating or preventing diabetes or its sequelae. Specific examples of such cultured models of diabetes include, but are not limited to, cultured rat endothelial cells from affected and nonaffected individuals (Bazan *et al.*, 1997, *Therapie 52:* 447-451), antigen-specific lymphocytes cultured from TCR-HA transgenic mice (Sarukhan *et al.*, 1998, *EMBO J. 17*: 71-80), the DAP.3Ag7 transfected mouse fibroblast cell line (Nabavieh *et al.*, 1998, *J. Autoimmun. 11:* 63-71), and white blood cell isolates from affected and non-affected human individuals (Elhadd *et al.*, 1997, *Q.J.M. 90*: 461-464).

In another embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing diabetes by administering the Therapeutic to a test animal exhibiting symptoms of diabetes, and measuring the change in said symptoms of diabetes after administration of said Therapeutic, wherein a reduction in the severity of the symptoms of, or prevention of, diabetes indicates that the Therapeutic has activity in treating or preventing diabetes. Such a test animal can be any one of a number of animal models known in the art for diabetes including, but not limited to, the non-obese diabetic mouse model of diabetes (Delovitch and Singh, 1997 *Immunity 7*: 727-738), TCR-HA transgenic mice (Sarukhan *et al.*, 1998, EMBO J. 17:71-80), IL-2-expressing transgenic mice (Elliot and Flavell, 1994, Int. Immunol. 6:1629-1637), and NOS2-expressing transgenic mice (Takamura *et al.*, 1998, J. Biol. Chem. 273:2493-2496).

Hearing Loss

In another specific embodiment of the present invention, a β-APP:HsLON complex is administered to treat or prevent hearing loss. HsLON has been implicated in both sensorineural hearing loss and maternally-inherited deafness. Accordingly, a β-APP:HsLON complex or a derivative, homolog, analog or fragment thereof, nucleic acids encoding the β-APP and HsLON proteins, anti-β-APP:HsLON antibodies, and other modulators of β-APP:HsLON complex activity or formation, can be tested for activity in treating or preventing hearing loss in *in vitro* and *in vivo* assays.

In one embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing hearing loss by contacting cultured cells that exhibit an indicator of decreased function *in vitro* with the Therapeutic, and comparing the level of said indicator in the cells contacted with the

Therapeutic, with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the Therapeutic has activity in treating or preventing hearing loss. Specific examples of such cultured models for hearing loss include, but are not limited to, cultured sensory epithelia from the mammalian inner ear (Holley and Lawlor, 1997, Audiol. Neurootol. 2:25-35), cultured auditory cortical neurons (Gopal and Gross, 1996, Acta Otolaryngol (Stockh) 116:690-696), and cultured spiral ganglia neurons (Marzella *et al.*, 1997, Neuroreport 8:1641-1644).

In another embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing hearing loss by administering the Therapeutic to a test animal that is predisposed to develop hearing loss, and measuring the change in said hearing loss after administration of said Therapeutic, wherein a reduction in the severity of the symptoms of, or prevention of, the hearing loss indicates that the Therapeutic has activity in treating or preventing hearing loss. Such a test animal can be any one of a number of animal models known in the art for hearing loss. These models include, but are not limited to, mouse (Steel and Brown, 1994, Trends Genet. 10:428-435) and human (Hughes, 1997, Audiol. Neurootol. 2:3-11) genetic models of hearing loss, aminoglycoside ototoxicity in cats (Leake *et al.*, 1997, Hear. Res. 113:117-132), and congenital deafness in Dalmatians (Niparko and Finger, 1997, Otolaryngol. Head Neck Surg. 117:229-235).

Male Infertility

In yet another specific embodiment of the present invention, a β-APP:HsLON complex is administered to treat or prevent male infertility. HsLON has been implicated in the development of male infertility. Accordingly, a β-APP:HsLON complex or a derivative, homolog, analog or fragment thereof, nucleic acids encoding the β-APP and HsLON proteins, anti-β-APP:HsLON antibodies, and other modulators of β-APP:HsLON complex activity or formation, can be tested for activity in treating or preventing male infertility in *in vitro* and *in vivo* assays.

In one embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing male infertility by contacting cultured cells that exhibit an indicator of male infertility *in vitro* with the Therapeutic, and comparing the level of said indicator in the cells contacted with the Therapeutic, with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the Therapeutic has activity in treating or preventing male infertility. Specific examples of such cultured models for male infertility include, but are not limited to, spermatogenic cell cultures from affected and unaffected individuals (Blanchard *et al.*, 1991, Mol. Reprod. Dev. 30: 275-282), cultured Leydig cells and macrophages from rat testes (Dirami *et al.*, 1991, J. Endocrinol. 130: 357-365), and cultured mammalian Sertoli cells (Syed *et al.*, 1997, J. Androl. 18: 264-273).

In another embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing male infertility by administering the Therapeutic to a test animal that is infertile, and measuring the change in said symptoms of the male infertility after administration of said Therapeutic, wherein a reduction in the severity of the symptoms of, or prevention of, the symptoms of male infertility indicates that the Therapeutic has activity in treating or preventing male infertility. Such a test animal can be any one of a number of animal models known in the art for male infertility including, but not limited to, mice expressing t haplotypes (Olds-Clarke, 1997, Rev. Reprod. 2:157-164), the syngeneic neonatal testicular graft model (Johnson *et al.*, 1997, Contracept. Fertil. Sex 25:549-555), Sertoli cell depleted rats (Orth *et al.*, 1988, Endocrinology 122:787-794), the aging male brown-Norway rat (Wang *et al.*, 1993, Endocrinology 133:2733-2781), and hybrid sterile mice Forejt, 1996, Trends Genet. 12:412-417). The applicability that these animal models have to future human therapeutics is well described in the review by Lamb and Niederberger, 1994, Urol. Clin. North Am. 21:377-387.

Diseases associates with mitochondrial DNA mutations

In a specific embodiment of the present invention, a β-APP:HsLON complex is administered to treat or prevent a disease or disorder associated with mitochondrial DNA mutations. HsLON has been implicated in a number of diseases or disorders associated with mitochondrial DNA mutations. Further, through the link between neurodegeneration and mitochondrial dysfunction, β-APP is also implicated in diseases of mitochondria. Accordingly, a β-APP:HsLON complex, nucleic acids encoding the β-APP and HsLON proteins, anti-β-APP:HsLON antibodies, and other modulators of β-APP:HsLON complex activity or formation, or derivatives, fragments, analogs or homologs thereof, can be tested for activity in treating or preventing a disease or disorder associated with mitochondrial DNA mutations in *in vitro* and *in vivo* assays.

In one embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing a disease or disorder associated with mitochondrial DNA mutation by contacting cultured cells that exhibit an indicator of a disease or disorder associated with mitochondrial DNA mutations *in vitro* with the Therapeutic, and comparing the level of said indicator in the cells contacted with the Therapeutic, with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the Therapeutic has activity in treating or preventing diseases or disorders associated with mitochondrial DNA mutations. Specific examples of such cultured models for diseases or disorders associated with mitochondrial DNA mutations include, but are not limited to, cultured myotubes from muscles of unaffected individuals and individuals with mitochondrial myopathies (Collombet *et al.*, 1996, Mol. Gen. Genet. 253:182-188), cultured human skin fibroblasts unaffected humans and from humans with diseases associated with mtDNA deletions (van de Corput *et al.*, 1997, J.

Histochem. Cytochem. 45:55-61), and cultured cell lines harboring various mtDNA mutations (Spelbrink *et al.*, 1997, Curr. Genet. 32:115-124).

In another embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing a disease or disorder associated with mitochondrial DNA mutation by administering the Therapeutic to a test animal having symptoms associated with mitochondrial DNA mutations, and measuring the change in said symptoms after administration of said Therapeutic, wherein a reduction in the severity of the symptoms of, or prevention of, the symptoms of the disease or disorder associated with mitochondrial DNA mutations, indicates that the Therapeutic has activity in treating or preventing a disease or disorder associated with mitochondrial DNA mutations. Such a test animal can be any one of a number of animal models known in the art for diseases or disorders associated with mitochondrial DNA mutations. These models include, but are not limited to, liver, heart, and brain tissues from adult and senescent rats (Gadaleta *et al.*, 1992, Mut. Res. 275:181-193), skeletal muscle tissue from adult and aging rhesus monkeys (Lee *et al.*, 1993, J. Gerontol. 48:B201-B205), and primate xenomitochondrial cybrids (Kenyon and Moraes, 1997, Proc. Natl. Acad. Sci. USA 94:9131-9135.

Modulation of β-APP:HsLON Complex Activity

The present invention provides methodologies relating to modulating the level or activity of a protein moiety that possesses the ability to participate in a β-APP:HsLON complex, via the administration of a binding partner of that protein (or derivative, fragment, analog or homolog thereof). β-APP (and derivatives, fragments, analogs and homologs thereof) may be assayed for its ability to modulate the activity or levels of HsLON by contacting a cell, or administering to an animal expressing the HsLON gene, with β-APP protein, or, alternatively, with a nucleic acid encoding β-APP or an antibody that immunospecifically-binds β-APP, or derivative, fragment, analog, or homolog thereof that contains the antibody binding domain, and measuring a change in HsLON levels or activity, wherein said change in HsLON levels or activity indicates that said β-APP possesses the ability to modulate HsLON levels or activity. In another embodiment, HsLON (and derivatives, fragments, analogs and homologs thereof) may be assayed for their ability to modulate the activity or levels of β-APP in an analogous manner.

Protein-Protein Interaction Assays

The present invention discloses methodologies for assaying and screening derivatives, fragments, analogs and homologs of HsLON for binding to β-APP. The derivatives, fragments, analogs and homologs of the HsLON that interact with β-APP may be identified by means of a yeast two hybrid assay system (see *e.g.*, Fields & Song, 1989. *Nature* 340: 245-246) or; preferably, a modification and

improvement thereof, as described in U.S. Patent Applications Serial Nos. 08/663,824 (filed June 14, 1996) and 08/874,825 (filed June 13, 1997), to Nandabalan, *et al.*, and that are incorporated by reference herein in their entireties.

The identification of interacting proteins by the improved yeast two hybrid system is based upon the detection of the expression of a reporter gene (hereinafter "Reporter Gene"), the transcription of which is dependent upon the reconstitution of a transcriptional regulator by the interaction of two proteins, each fused to one half of the transcriptional regulator. The bait β-APP (or derivative, fragment, analog or homolog) and prey protein (proteins to be tested for ability to interact with the bait protein) are expressed as fusion proteins to a DNA-binding domain, and to a transcriptional regulatory domain, respectively, or *vice versa*. In a specific embodiment of the present invention, the prey population may be one or more nucleic acids encoding mutants of HsLON (*e.g.*, as generated by site-directed mutagenesis or another method of producing mutations in a nucleotide sequence). The prey populations are proteins encoded by DNA (*e.g.*, cDNA, genomic DNA or synthetically generated DNA), said DNAs derived either from a specific gene of choice, or from cDNA libraries obtain from a cell type of interest. For example, the populations may be expressed from chimeric genes comprising cDNA sequences derived from a non-characterized sample of a population of cDNA from mammalian RNA. In another specific embodiment, recombinant biological libraries expressing random peptides may be used as the source of prey nucleic acids.

The present invention discloses methods for the screening for inhibitors of HsLON. In brief, the protein-protein interaction assay may be performed as previously described herein, with the exception that it is performed in the presence of one or more candidate molecules. A resulting alteration in level of Reporter Gene activity, in relation to that level which was present when the one or more candidate molecules are absent, indicates that the candidate molecule exerts an effect on the interacting pair. In a preferred embodiment, inhibition of the protein interaction is necessary for the yeast cells to survive, for example, where a non-attenuated protein interaction causes the activation of the *URA3* gene, causing yeast to die in medium containing the chemical 5-fluoroorotic acid. See *e.g.*, Rothstein, 1983. *Meth. Enzymol.* 101: 167-180.

In general, the proteins comprising the bait and prey populations are provided as fusion (chimeric) proteins, preferably by recombinant expression of a chimeric coding sequence containing each protein contiguous to a pre-selected sequence. For one population, the pre-selected sequence is a DNA-binding domain that may be any DNA-binding domain, so long as it specifically recognizes a DNA sequence within a promoter (*e.g.*, a transcriptional activator or inhibitor). For the other population, the pre-selected sequence is an activator or inhibitor domain of a transcriptional activator or inhibitor, respectively. The regulatory domain alone (not as a fusion to a protein sequence) and the

DNA-binding domain alone (not as a fusion to a protein sequence), preferably, do not detectably interact, so as to avoid false-positives in the assay. The assay system further includes a reporter gene operably linked to a promoter that contains a binding site for the DNA-binding domain of the transcriptional activator (or inhibitor). Accordingly, in the practice of the present invention, the binding of β-APP fusion protein to a prey fusion protein leads to reconstitution of a transcriptional activator (or inhibitor), which concomitantly alters expression of the Reporter Gene.

In a specific embodiment, the present invention discloses a methodology for detecting one or more protein-protein interactions comprising the following steps: (*i*) recombinantly-expressing β-APP (or a derivative, fragment, analog or homolog thereof) in a first population of yeast cells of a first mating type and possessing a first fusion protein containing β-APP sequence and a DNA-binding domain; wherein said first population of yeast cells contains a first nucleotide sequence operably-linked to a promoter that is "driven" by one or more DNA-binding sites recognized by said DNA-binding domain such that an interaction of said first fusion protein with a second fusion protein (comprising a transcriptional activation domain) results in increased transcription of said first nucleotide sequence; (*ii*) negatively selecting to eliminate those yeast cells in said first population in which said increased transcription of said first nucleotide sequence occurs in the absence of said second fusion protein; (*iii*) recombinantly expressing in a second population of yeast cells of a second mating type different from said first mating type, a plurality of said second fusion proteins; wherein said second fusion protein is comprised of a sequence of a derivative, fragment, analog or homolog of a HsLON and an activation domain of a transcriptional activator, in which the activation domain is the same in each said second fusion protein; (*iv*) mating said first population of yeast cells with said second population of yeast cells to form a third population of diploid yeast cells, wherein said third population of diploid yeast cells contains a second nucleotide sequence operably linked to a promoter "driven" by a DNA-binding site recognized by said DNA-binding domain such that an interaction of a first fusion protein with a second fusion protein results in increased transcription of said second nucleotide sequence, in which the first and second nucleotide sequences can be the same or different and (*v*) detecting said increased transcription of said first and/or second nucleotide sequence, thereby detecting an interaction between a first fusion protein and a second fusion protein.

In a preferred embodiment, the bait (a β-APP sequence) and the prey (a library of chimeric genes) are combined by mating the two yeast strains on solid media for a period of approximately 6-8 hours. In a less preferred embodiment, the mating is performed in liquid media. The resulting diploids contain both types of chimeric genes (*i.e.*, the DNA-binding domain fusion and the activation domain fusion). After an interactive population is obtained, the DNA sequences encoding the pairs of interactive proteins are isolated by a method wherein either the DNA-binding domain hybrids or the

activation domain hybrids are amplified, in separate reactions. Preferably, the amplification is carried out by polymerase chain reaction (PCR; see *e.g.*, Innis, *et al.*, 1990. PCR PROTOCOLS, Academic Press, Inc., San Diego, CA) utilizing pairs of oligonucleotide primers specific for either the DNA-binding domain hybrids or the activation domain hybrids. The PCR amplification reaction may also be performed on pooled cells expressing interacting protein pairs, preferably pooled arrays of interactants. Other amplification methods known within the art may also be used including, but not limited to, ligase chain reaction; Qβ-replicase or the like. See *e.g.*, Kricka, *et al.*, 1995. MOLECULAR PROBING, BLOTTING, AND SEQUENCING, Academic Press, New York, NY.

In an additional embodiment of the present invention, the plasmids encoding the DNA-binding domain hybrid and the activation domain hybrid proteins may also be isolated and cloned by any of the methods well-known within the art. For example, but not by way of limitation, if a shuttle vector (for *e.g.*, yeast to *E. coli*) is used to express the fusion proteins, the genes may be subsequently recovered by transforming the yeast DNA into *E. coli* and recovering the plasmids from the bacteria. See *e.g.*, Hoffman, *et al.*, 1987 *Gene* 57: 267-272.

Pharmaceutical Compositions

The invention provides methods of treatment and prophylaxis by the administration to a subject of a pharmaceutically-effective amount of a Therapeutic of the invention. In a preferred embodiment, the Therapeutic is substantially purified and the subject is a mammal, and most preferably, human.

Formulations and methods of administration that can be employed when the Therapeutic comprises a nucleic acid are described *supra*. Various delivery systems are known and can be used to administer a Therapeutic of the present invention including, but not limited to: (*i*) encapsulation in liposomes, microparticles, microcapsules; (*ii*) recombinant cells capable of expressing the Therapeutic; (*iii*) receptor-mediated endocytosis (see, *e.g.*, Wu & Wu, 1987. *J. Biol. Chem.* 262: 4429-4432); (*iv*) construction of a Therapeutic nucleic acid as part of a retroviral or other vector, and the like.

Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The Therapeutics of the present invention may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically-active agents. Administration can be systemic or local. In addition, it may be advantageous to administer the Therapeutic into the central nervous system by any suitable route, including intraventricular and intrathecal injection. Intraventricular injection may be facilitated by an intraventricular catheter attached

to a reservoir (*e.g.*, an Ommaya reservoir). Pulmonary administration may also be employed by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. It may also be desirable to administer the Therapeutic locally to the area in need of treatment. This may be achieved by, for example, and not by way of limitation, by local infusion during surgery, by topical application, by injection, by means of a catheter, by means of a suppository, or by means of an implant. In a specific embodiment, administration may be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

In another embodiment of the present invention, the Therapeutic may be delivered in a vesicle, in particular a liposome. See *e.g.*, Langer, 1990. *Science* 249: 1527-1533. In yet another embodiment, the Therapeutic can be delivered in a controlled release system including, but not limited to, a delivery pump (see *e.g.*, Saudek, *et al.*, 1989. *New Engl. J. Med.* 321: 574) and a semi-permeable polymeric material (see *e.g.*, Howard, *et al.*, 1989. *J. Neurosurg.* 71: 105). Additionally, the controlled release system can be placed in proximity of the therapeutic target (*e.g.*, the brain), thus requiring only a fraction of the systemic dose. See, *e.g.*, Goodson, In: MEDICAL APPLICATIONS OF CONTROLLED RELEASE, CRC Press, Bocca Raton, FL (1984).

In a specific embodiment, where the Therapeutic is a nucleic acid encoding a protein, the Therapeutic nucleic acid may be administered *in vivo* to promote expression of its encoded protein by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular (*e.g.*, via a retroviral vector, direct injection, use of microparticle bombardment, coating with lipids or cell-surface receptors or transfecting agents, or administering it in linkage to a homeobox-like peptide that is known to enter the nucleus (see *e.g.*, Joliot, *et al.*, 1991. *Proc. Natl. Acad. Sci. USA* 88: 1864-1868), and the like. Alternatively, a nucleic acid Therapeutic can be introduced intracellularly and incorporated into host cell DNA for expression, *e.g.*, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically-effective amount of Therapeutic, and a pharmaceutically acceptable carrier. As utilized herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government, or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals and, more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered and includes, but is not limited, to such sterile liquids as water and oils.

The amount of the Therapeutic of the invention that will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and may be determined by standard clinical techniques by those of average skill within the art. In addition, *in vitro*

assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration and the overall seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and of each patient's circumstances. However, suitable dosage ranges for intravenous administration of a Therapeutics herein are generally about 20-500 micrograms (μg) of active compound per kilogram (kg) body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 picograms (pg)/kg body weight to 1 milligram (mg)/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems. Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The present invention also provides a pharmaceutical pack or kit, comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions and Therapeutics of the present invention. Optionally associated with such container(s) may be a notice in the form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceutical or biological products, which notice reflects approval by the agency of manufacture, use, or sale for human administration.

SPECIFIC EXAMPLES

Example 1: Identification and specificity of β-APP:HsLON interaction

A modified, improved yeast two hybrid system was used to identify protein interactions for the neuronal protein product β-APP. Yeast is an eukaryote, and therefore any intermolecular protein interactions detected in this system are likely to occur under physiological conditions found in mammalian cells (Chien *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:9578-9581). Expression vectors were constructed to encode two hybrid proteins. One hybrid consists of the DNA binding domain of the yeast transcriptional activator Gal4 fused to a predetermined "bait" polypeptide. The other hybrid consisted of the Gal4 activator domain fused to "prey" polypeptide sequences encoded by a mammalian cDNA library. In a "reverse" screen, the bait was fused to the activator domain, and the prey was fused to the DNA binding domain, but the assay was otherwise identically performed. In a matrix-mating assay, β-APP, HsLON, and other proteins were inserted into complementary (a and alpha) mating types of yeast using methods known in the art. Mating was carried out to express both vector constructs within the same yeast cells, thus allowing interaction to occur. Interaction between the domains led to transcriptional activation of reporter genes containing *cis*-binding elements for Gal4. The reporter genes encoding the indicator protein β-galactosidase, and metabolic markers for uracil and histidine auxotrophy, were included in specific fashion in one or the other of the yeast strains used in the mating.

In this way, yeast were selected for successful mating, expression of both fusion constructs, and expression of β-APP-interacting proteins and the interaction of both fusion proteins.

The HsLON cDNA were obtained from a commercial fetal brain cDNA library of 3.5×10^6 independent isolates (Clontech #HL4029AH, Palo Alto, CA). The library was synthesized from Xho 1-dT_{15} primed fetal brain mRNA (from five male/female 19-22 week fetuses) that was directionally cloned into pACT2, a yeast Gal4 activation domain cloning vector including the LEU2 gene for selection in yeast deficient in leucine biosynthesis.

Interaction of the prey cDNA products was tested against an array of 18 bait proteins, one of which was encoded by the β-APP gene sequence from nucleotide 1935 to 2060, which encodes $\beta\text{-APP}_{695}$ residues 597-638, commonly known as the 42 residue peptide β-A4(42). The bait fragment was amplified from the Clontech pACT2 library (Clontech, Palo Alto, CA) by polymerase chain reaction (PCR) using the forward primer 5'GATGCAGAATTCCGACATGACTCAG3' (SEQ ID NO:5) and the reverse primer 5'ATGGTGGGCGGTGTTGTCATAGCG3' (SEQ ID NO:6) by standard techniques. The fragment was cloned into the *SfiI* site of the vector pAS-SfiI, constructed by introducing an *SfiI*-containing polylinker into the vector pAS2-1 (Clontech, Palo Alto, CA). This vector is a yeast DNA-binding domain cloning vector that contains the *TRP1* gene for selection in yeast strains deficient in tryptophan biosynthesis. The bait sequence was confirmed by nucleic acid sequencing to confirm that PCR amplification reproduced an accurate copy of the β-APP sequence (Figure 1, SEQ ID NO:1). This test determined that, as predicted, the bait sequence encoded an interacting domain identical to the human β-A4 peptide of 42 amino acids.

Example 2: Test for the specificity of β-APP:HsLON interaction

β-APP was transformed by lithium acetate/polyethylene glycol transformation (Ito *et al.*, 1983, *J. Bacteriol.* 153: 163-168) into the yeast strain N106' (mating type a, *ura3, his3, ade2, trp1, leu2, gal4, gal80, cyh'*, *Lys2::*$GAL1_{UAS}$-$HIS3_{TATA}$-*HIS3, ura 3::*$GAL1_{UAS}$-GAL_{TATA}-*lacZ*), while the coding sequences of HsLON were transformed into the yeast strain YULH (mating type alpha, *ura3, his3, lys2, trp1, leu2, gal4, gal80, GAL1-URA3*). The two transformed populations were then mated using standard methods in the art. Sherman *et al.*, eds., 1991, GETTING STARTED WITH YEAST, Vol. 194, Academic Press, New York. Briefly, cells were grown until mid-to-late log phase on media that selected for the presence of the appropriate plasmids. The two mating strains, alpha and a, were then, diluted in YAPD media, filtered onto nitrocellulose membranes, and incubated at 30° C for 6-8 hours. The cells were then transferred to media selective for the desired diploids, *i.e.*, yeast harboring reporter genes for β-galactosidase, uracil auxotrophy, and histidine auxotrophy, and expression of the vectors encoding the bait and prey. The mating products were plated on SC (synthetic complete) (Sambrook *et al.*, 1989, A

LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Press, New York) media lacking adenine and lysine (to select for successful mating), leucine and tryptophan (to select for expression of genes encoded by both plasmids), and uracil and histidine (to select for protein interactions). This medium is herein referred to as SCS medium, for SC Selective medium.

Selected clones were tested for expression of β-galactosidase to confirm the formation of a β-APP:HsLON complex. Filter-lift β-galactosidase assays were performed as modified from the protocol of Breeden and Nasmyth, 1985, *Cold Spring Harbor Quant. Biol.* 50: 643-650. Colonies were patched onto SCS plates, grown overnight, and replica plated onto nitrocellulose filters. The filters were then assayed for β-galactosidase activity as per Breeden and Nasmyth, 1985, *Cold Spring Harbor Quant. Biol.* 50: 643-650. Colonies that were positive turned a visible blue.

To test for the specificity of β-APP:HsLON interaction, two general tests were first performed. In the first instance, YULH cells expressing HsLON were created and plated on SC-Ura plates, grown for 1-2 days, and examined for growth. No growth was found for HsLON, confirming that it is not a "self-activating" protein, that is, HsLON requires interaction with a second protein domain for a functional activation complex. In the second instance, plasmids containing β-APP inserts were transformed into strain N106^r (mating type alpha) and mated with yeast strain YULH (mating type a) expressing either CDK2, HsLON, or certain other proteins. Promiscuous binders, that is, insert products able to bind with many other proteins in a non-specific fashion, would interact non-specifically with non-CDK2 domains, and would be discarded as non-specific interactants. As illustrated in Figure 3, the intersection of the β-APP column with the HsLON row indicates growth (Box A) (*i.e.*, a positive interaction). In contrast, the intersection of the β-APP column with the rows for P1 – P5, and the intersection of the HsLON row with columns for B1 – B6, indicates no growth, *i.e.*, no protein interaction. These data demonstrate the specificity of the β-APP:HsLON interaction.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entirety.

EQUIVALENTS

From the foregoing detailed description of the specific embodiments of the invention, it should be apparent that unique compositions and methods of use for β-APP, HsLON, and β-APP:HsLON complexes have been described. Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims which follow. In particular, it is contemplated by the inventor that various substitutions, alterations, and modifications may be made to the invention without departing from the spirit and scope of the invention as defined by the claims. For instance, the choice of disease states in which β-APP, HsLON, and β-APP:HsLON complexes provide utility through diagnosis, screening, treatment of various diseases and disorders is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein.

WHAT IS CLAIMED IS:

1. A purified complex of β-APP and HsLON.

2. The purified complex of claim 1 in which the proteins are human proteins.

3. A purified complex selected from the group consisting of a complex of a derivative of β-APP and HsLON, a complex of β-APP and a derivative of HsLON, and a complex of a derivative of β-APP and a derivative of HsLON; in which the derivative of β-APP is able to form a complex with a wild-type HsLON and the derivative of HsLON is able to form a complex with wild-type β-APP.

4. The purified complex of claim 3 in which the derivative of β-APP or HsLON is fluorescently labeled.

5. A chimeric protein comprising a fragment of β-APP consisting of at least 6 amino acids fused via a covalent bond to a fragment of HsLON consisting of at least 6 amino acids.

6. The chimeric protein of claim 5 in which the fragment of β-APP is a fragment capable of binding HsLON and in which the fragment of HsLON is a fragment capable of binding β-APP.

7. The chimeric protein of claim 6 in which the fragment of β-APP and the fragment of HsLON form a β-APP:HsLON complex.

8. An antibody which immunospecifically binds the complex of claim 1 or a fragment or derivative of said antibody containing the binding domain thereof.

9. The antibody of claim 8 which does not immunospecifically bind β-APP or HsLON that is not part of a β-APP:HsLON complex.

10. An isolated nucleic acid or an isolated combination of nucleic acids comprising a nucleotide sequence encoding β-APP and a nucleotide sequence encoding HsLON.

11. The isolated nucleic acid or isolated combination of nucleic acids of claim 10 which are nucleic acid vectors.

12. The isolated nucleic acid or isolated combination of nucleic acids of claim 11 in which the β-APP coding sequence and the HsLON coding sequence are operably linked to a promoter.

13. An isolated nucleic acid that comprises a nucleotide sequence encoding the chimeric protein of claim 7.

14. A cell containing a nucleic acid of claim 10, which nucleic acid is recombinant.

15. A cell containing a nucleic acid of claim 12, which nucleic acid is recombinant.

16. A recombinant cell containing a nucleic acid of claim 15, which nucleic acid is recombinant.

17. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the complex of claim 1; and a pharmaceutically acceptable carrier.

18. The pharmaceutical composition of claim 17 in which the proteins are human proteins.

19. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the complex of claim 3; and a pharmaceutically acceptable carrier.

20. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the chimeric protein of claim 5; and a pharmaceutically acceptable carrier.

21. A pharmaceutical composition of comprising a therapeutically or prophylactically effective amount of the chimeric protein of claim 6; and a pharmaceutically acceptable carrier.

22. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the antibody of claim 8 or a fragment or derivative of said antibody containing the binding domain thereof; and a pharmaceutically acceptable carrier.

23. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the antibody of claim 9 or a fragment or derivative of said antibody containing the binding domain thereof; and a pharmaceutically acceptable carrier.

24. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the nucleic acid or combination of nucleic acids of claim 10; and a pharmaceutically acceptable carrier.

25. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the isolated nucleic acid of claim 13; and a pharmaceutically acceptable carrier.

26. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the recombinant cell of claim 14; and a pharmaceutically acceptable carrier.

27. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the protein of claim 15; and a pharmaceutically acceptable carrier.

28. A method of producing a complex of β-APP and HsLON comprising growing a recombinant cell containing the nucleic acid of claim 10 such that the encoded β-APP and HsLON proteins are expressed and bind to each other, and recovering the expressed complex of β-APP and HsLON.

29. A method of diagnosing or screening for the presence of or a predisposition for developing a disease or disorder characterized by an aberrant level of a complex of β-APP and HsLON, in a subject comprising measuring the level of said complex, RNA encoding β-APP and HsLON, or functional activity of said complex in a sample derived from the subject, in which an increase or decrease in the level of said complex, said RNA encoding β-APP and HsLON, or functional activity of said complex in the sample, relative to the level of said complex, said RNA encoding β-APP and HsLON or functional activity of said complex found in an analogous sample not having the disease or disorder or a predisposition for developing the disease or disorder, indicates the presence of the disease or disorder or a predisposition for developing the disease or disorder.

30. A kit comprising in one or more containers a substance selected from the group consisting of a complex of β-APP and HsLON, an antibody against said complex, nucleic acid probes capable of hybridizing to RNA of β-APP and RNA of HsLON, or pairs of nucleic acid primers capable of priming amplification of at least a portion of the β-APP gene and the HsLON gene.

31. A method of treating or preventing a disease or disorder involving aberrant levels of a complex of β-APP and HsLON, in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule or molecules that modulate the function of said complex.

32. The method of claim 31 in which said disease or disorder involves decreased levels of said complex and said molecule or molecules promote the function of the complex of β-APP and HsLON and are selected from the group consisting of a complex of β-APP and HsLON; a derivative or analog of a complex of β-APP and HsLON, which complex is more stable or more active than the wild type complex; nucleic acids encoding β-APP and HsLON proteins; and nucleic acids encoding a derivative or analog of β-APP and HsLON that form a complex that is more stable or more active than the wild type complex.

33. The method of claim 31 in which said disease or disorder involves increased levels of said complex and said molecule or molecules inhibit the function of said complex and are selected from the group consisting of an antibody against said complex or a fragment or derivative thereof containing the binding region thereof; β-APP and HsLON antisense nucleic acids; and nucleic acids comprising at least a portion of the β-*APP and* the *HsLON* gene into which a heterologous nucleotide sequence has been inserted such that said heterologous sequence inactivates the biological activity of the at least a portion of the β-*APP and HsLON* genes, in which the β-*APP and* the *HsLON* gene portions flank the heterologous sequences so as to promote homologous recombination with genomic β-*APP and HsLON* genes.

34. A method of treating or preventing a disease or disorder involving an aberrant level of HsLON in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of HsLON.

35. The method of claim 34 in which said disease or disorder involves a decreased level of HsLON and said molecule promotes the function of HsLON and is selected from the group consisting of the HsLON protein, derivative or analog of HsLON that is active in binding β-APP, a nucleic acid encoding HsLON, and a nucleic acid encoding a derivative or analog of HsLON that is active in binding β-APP.

36. The method of claim 34 in which said disease or disorder involves an increased level of HsLON and said molecule inhibits HsLON function and is selected from the group consisting of an anti-HsLON antibody or a fragment or derivative thereof containing the binding region thereof, a *HsLON* antisense nucleic acid, and a nucleic acid comprising at least a portion of the *HsLON* gene into which a heterologous nucleotide sequence has been inserted such that said heterologous sequence inactivates the biological activity of the at least a portion of the *HsLON* gene, in which the *HsLON* gene portion flanks the heterologous sequence so as to promote homologous recombination with the genomic *HsLON* gene.

37. A method of treating or preventing a disease or disorder involving an aberrant level of β-APP in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of β-APP.

38. The method of claim 37 in which said disease or disorder involves a decreased level of β-APP and said molecule promotes the function of β-APP and is selected from the group consisting of the β-APP protein, derivative or analog of β-APP that is active in binding HsLON, a nucleic acid encoding β-APP, and a nucleic acid encoding a derivative or analog of β-APP that is active in binding HsLON.

39. The method of claim 37 in which said disease or disorder involves an increased level of β-APP and said molecule inhibits β-APP function and is selected from the group consisting of an anti-β-APP antibody or a fragment or derivative thereof containing the binding region thereof, a β-APP antisense nucleic acid, and a nucleic acid comprising at least a portion of the β-*APP* gene into which a heterologous nucleotide sequence has been inserted such that said heterologous sequence inactivates the biological activity of the at least a portion of the β-*APP* gene, in which the β-*APP* gene portion flanks the heterologous sequence so as to promote homologous recombination with the genomic β-*APP* gene.

40. A method for screening a purified complex of β-APP and HsLON, or a derivative of said complex, or a modulator of the activity of said complex for activity in treating or preventing neurodegenerative disease comprising contacting cultured cells that exhibit an indicator of neurodegenerative disease *in vitro* with said complex, derivative or modulator; and comparing the level of said indicator in the cells contacted with the complex, derivative, or modulator with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the complex, derivative or modulator has activity in treating or preventing neurodegenerative disease.

41. A method for screening a purified complex of β-APP and HsLON, or a derivative of said complex, or a modulator of the activity of said complex for activity in treating or preventing an cardiomyopathy comprising contacting cultured cells that exhibit an indicator of a cardiomyopathy *in vitro* with said complex, derivative or modulator; and comparing the level of said indicator in the cells contacted with the complex, derivative, or modulator with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the complex, derivative or modulator has activity in treating or preventing cardiomyopathy.

42. A method for screening a purified complex of β-APP and HsLON, or a derivative of said complex, or a modulator of the activity of said complex for activity in treating or preventing a diabetes comprising contacting cultured cells that exhibit an indicator of a diabetes *in vitro* with said complex, derivative or modulator; and comparing the level of said indicator in the cells contacted with the complex, derivative, or modulator with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the complex, derivative or modulator has activity in treating or preventing diabetes.

43. A method for screening a purified complex of β-APP and HsLON, or a derivative of said complex, or a modulator of the activity of said complex for activity in treating or preventing a hearing loss comprising contacting cultured cells that exhibit an indicator of a hearing loss *in vitro* with said complex, derivative or modulator; and comparing the level of said indicator in the cells contacted with the complex, derivative, or modulator with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the complex, derivative or modulator has activity in treating or preventing hearing loss.

44. A method for screening a purified complex of β-APP and HsLON, or a derivative of said complex, or a modulator of the activity of said complex for activity in treating or preventing male infertility comprising contacting cultured cells that exhibit an indicator of a male infertility *in vitro* with said complex, derivative or modulator; and comparing the level of said indicator in the cells contacted with the complex, derivative, or modulator with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the complex, derivative or modulator has activity in treating or preventing male infertility.

45. A method for screening a purified complex of β-APP and HsLON, or a derivative of said complex, or a modulator of the activity of said complex for activity in treating or preventing mitochondrial DNA mutation associated disorders comprising contacting cultured cells that exhibit an indicator of a mitochondrial DNA mutation associated disorders *in vitro* with said complex, derivative or modulator; and comparing the level of said indicator in the cells contacted with the complex, derivative, or modulator with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the complex, derivative or modulator has activity in treating or preventing mitochondrial DNA mutation associated disorders.

46. A method of screening for a molecule that modulates directly or indirectly the formation of a complex of β-APP and HsLON comprising measuring the levels of said complex formed from β-APP and HsLON proteins in the presence of said molecule under conditions conducive to formation of the complex; and comparing the levels of said complex with the levels of said complex that are formed in the absence of said molecule, wherein a lower or higher level of said complex in the presence of said molecule indicates that the molecule modulates formation of said complex.

47. A recombinant non-human animal in which both an endogenous β-*APP* gene and an endogenous *HsLON* have been deleted or inactivated by homologous recombination or insertional mutagenesis of said animal or an ancestor thereof.

48. A recombinant non-human animal containing both a β-*APP* gene and a *HsLON* gene, in which the β-*APP* gene is under the control of a promoter that is not the native β-*APP* gene promoter and the *HsLON* gene is under the control of a promoter that is not the native *HsLON* gene promoter.

49. A recombinant non-human animal containing a transgene comprising a nucleic acid sequence encoding the chimeric protein of claim 7.

50. A method of modulating the activity or levels of β-APP by contacting a cell with, or administering an animal expressing a β-*APP* gene, a HsLON protein, or a nucleic acid encoding said protein or an antibody that immunospecifically binds said protein or a fragment or derivative of said antibody containing the binding domain thereof.

51. A method of modulating the activity or levels of HsLON by contacting a cell with, or administering an animal expressing a gene encoding said protein, β-APP, or a nucleic acid encoding β-APP, or an antibody that immunospecifically binds β-APP or a fragment or derivative of said antibody containing the binding domain thereof.

52. A method of modulating the activity or levels of a complex of β-APP and HsLON by contacting a cell with, or administering an animal expressing and forming said complex, a molecule that modulates the formation of said complex.

53. A method for identifying a molecule that modulates activity of β-APP or HsLON or a complex of β-APP and HsLON comprising contacting one or more candidate molecules with β-APP in the presence of HsLON; and measuring the amount of complex that forms between β-APP and HsLON; wherein an increase or decrease in the amount of complex that forms relative to the amount that forms in the absence of the candidate molecules indicates that the molecules modulate the activity of β-APP or HsLON or said complex of β-APP and HsLON.

54. The method of claim 53 wherein said contacting is carried out by administering the candidate molecules to the recombinant non-human animal of claim 49.

55. The method of claim 54 wherein said contacting is carried out *in vitro*; and β-APP, HsLON, and said candidate molecules are purified.

56. A method for screening a derivative or analog of β-APP for biological activity comprising contacting said derivative or analog of β-APP with HsLON; and detecting the formation of a complex between said derivative or analog of β-APP and HsLON; wherein detecting formation of said complex indicates that said derivative or analog of β-APP has biological activity.

57. A method for screening a derivative or analog of HsLON for biological activity comprising contacting said derivative or analog of HsLON with β-APP; and detecting the formation of a complex between said derivative or analog of HsLON and β-APP; wherein detecting the formation of said complex indicates that said derivative or analog of HsLON has biological activity.

58. A method of monitoring the efficacy of a treatment of a disease or disorder characterized by an aberrant level of a complex of β-APP and HsLON in a subject administered said treatment for said disease or disorder comprising measuring the level of said complex, RNA encoding β-APP and HsLON, or functional activity of said complex in a sample derived from said subject wherein said sample is taken from said subject after the administration of said treatment and compared to (a) said level in a sample taken from said subject prior to the administration of the treatment or (b) a standard level associated with the pretreatment stage of the disease or disorder, in which the change, or lack of change in the level of said complex, said RNA encoding β-APP and HsLON, or functional activity of said complex in said sample taken after the administration of said treatment relative to the level of said complex, said RNA encoding β-APP and HsLON or functional activity of said complex in said sample taken before the administration of said treatment or to said standard level indicates whether said administration is effective for treating said disease or disorder.

59. A method of treating or preventing neurodegeneration in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of a complex of β-APP and HsLON.

60. A method of treating or preventing cardiomyopathy in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of a complex of β-APP and HsLON.

61. A method of treating or preventing diabetes in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of a complex of β-APP and HsLON.

62. A method of treating or preventing hearing loss in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of a complex of β-APP and HsLON.

63. A method of treating or preventing male infertility or an associated disease in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of a complex of β-APP and HsLON.

64. A method of treating or preventing mitochondrial DNA mutation or an associated disease in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of a complex of β-APP and HsLON.

FIGURE 1

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AGTTTCCTCG GCAGCGGTAG GCGAGAGCAC GCGGAGGAGC GTGCGCGGGG CCCCGGGAGA  60
CGGCGGCGGT GGCGGCGCGG GCAGAGCAAG GACGCGGCGG ATCCCACTCG CACAGCAGCG 120
CACTCGGTGC CCCGCGCAGG GTCGCG   146

ATG CTG CCC GGT TTG GCA CTG CTC CTG CTG GCC GCC TGG ACG GCT        191
Met Leu Pro Gly Leu Ala Leu Leu Leu Ala Ala Trp Thr Ala
1               5                  10                  15

CGG GCG CTG GAG GTA CCC ACT GAT GGT AAT GCT GGC CTG CTG GCT        236
Arg Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala
                20                  25                  30

GAA CCC CAG ATT GCC ATG TTC TGT GGC AGA CTG AAC ATG CAC ATG        281
Glu Pro Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met
                35                  40                  45

AAT GTC CAG AAT GGG AAG TGG GAT TCA GAT CCA TCA GGG ACC AAA        326
Asn Val Gln Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys
                50                  55                  60

ACC TGC ATT GAT ACC AAG GAA GGC ATC CTG CAG TAT TGC CAA GAA        371
Thr Cys Ile Asp Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu
                65                  70                  75

GTC TAC CCT GAA CTG CAG ATC ACC AAT GTG GTA GAA GCC AAC CAA        416
Val Tyr Pro Glu Lue Gln Ile Thr Asn Val Val Glu Ala Asn Gln
                80                  85                  90

CCA GTG ACC ATC CAG AAC TGG TGC AAG CGG GGC CGC AAG CAG TGC        461
Pro Val Thr Ile Gln Asn Trp Cys Lys Arg Gly Arg Lys Gln Cys
                95                 100                 105

AAG ACC CAT CCC CAC TTT GTG ATT CCC TAC CGC TGC TTA GTT GGT        506
Lys Thr His Pro His Phe Val Ile Pro Tyr Arg Cys Leu Val Gly
               110                 115                 120

GAG TTT GTA AGT GAT GCC CTT CTC GTT CCT GAC AAG TGC AAA TTC        551
Glu Phe Val Ser Asp Ala Leu Leu Val Pro Asp Lys Cys Lys Phe
               125                 130                 135

TTA CAC CAG GAG AGG ATG GAT GTT TGC GAA ACT CAT CTT CAC TGG        596
Leu His Gln Glu Arg Met Asp Val Cys Glu Thr His Leu His Trp
               140                 145                 150

CAC ACC GTC GCC AAA GAG ACA TGC AGT GAG AAG AGT ACC AAC TTG        641
His Thr Val Ala Lys Glu Thr Cys Ser Glu Lys Ser Thr Asn Leu
               155                 160                 165

CAT GAC TAC GGC ATG TTG CTG CCC TGC GGA ATT GAC AAG TTC CGA        686
His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile Asp Lys Phe Arg
               170                 175                 180

GGG GTA GAG TTT GTG TGT TGC CCA CTG GCT GAA GAA AGT GAC AAT        731
Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu Ser Asp Asn
               185                 190                 195

GTG GAT TCT GCT GAT GCG GAG GAG GAT GAC TCG GAT GTC TGG TGG        776
Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val Trp Trp
               200                 205                 210
```

FIGURE 1 (CONT.)

```
GGC GGA GCA GAC ACA GAC TAT GCA GAT GGG AGT GAA GAC AAA GTA        821
Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys Val
                215                 220                 225

GTA GAA GTA GCA GAG GAG GAA GAA GTG GCT GAG GTG GAA GAA GAA        866
Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
                230                 235                 240

GAA GCC GAT GAT GAC GAG GAC GAT GAG GAT GGT GAT GAG GTA GAG        911
Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu
                245                 250                 255

GAA GAG GCT GAG GAA CCC TAC GAA GAA GCC ACA GAG AGA ACC ACC        956
Glu Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr
                260                 265                 270

AGC ATT GCC ACC ACC ACC ACC ACC ACC ACA GAG TCT GTG GAA GAG       1001
Ser Ile Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu
                275                 280                 285

GTG GTT CGA GTT CCT ACA ACA GCA GCC AGT ACC CCT GAT GCC GTT       1046
Val Val Arg Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val
                290                 295                 300

GAC AAG TAT CTC GAG ACA CCT GGG GAT GAG AAT GAA CAT GCC CAT       1091
Asp Lys Tyr Leu Glu Thr Pro Gly Asp Glu Asn Glu His Ala His
                305                 310                 315

TTC CAG AAA GCC AAA GAG AGG CTT GAG GCC AAG CAC CGA GAG AGA       1136
Phe Gln Lys Ala Lys Glu Arg Leu Glu Ala Lys His Arg Glu Arg
                320                 325                 330

ATG TCC CAG GTC ATG AGA GAA TGG GAA GAG GCA GAA CGT CAA GCA       1181
Met Ser Gln Val Met Arg Glu Trp Glu Glu Ala Glu Arg Gln Ala
                335                 340                 345

AAG AAC TTG CCT AAA GCT GAT AAG AAG GCA GTT ATC CAG CAT TTC       1226
Lys Asn Leu Pro Lys Ala Asp Lys Lys Ala Val Ile Gln His Phe
                350                 355                 360

CAG GAG AAA GTG GAA TCT TTG GAA CAG GAA GCA GCC AAC GAG AGA       1271
Gln Glu Lys Val Glu Ser Leu Glu Gln Glu Ala Ala Asn Glu Arg
                365                 370                 375

CAG CAG CTG GTG GAG ACA CAC ATG GCC AGA GTG GAA GCC ATG CTC       1316
Gln Gln Leu Val Glu Thr His Met Ala Arg Val Glu Ala Met Leu
                380                 385                 390

AAT GAC CGC CGC CGC CTG GCC CTG GAG AAC TAC ATC ACC GCT CTG       1361
Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn Tyr Ile Thr Ala Leu
                395                 400                 405

CAG GCT GTT CCT CCT CGG CCT CGT CAC GTG TTC AAT ATG CTA AAG       1406
Gln Ala Val Pro Pro Arg Pro Arg His Val Phe Asn Met Leu Lys
                410                 415                 420

AAG TAT GTC CGC GCA GAA CAG AAG GAC AGA CAG CAC ACC CTA AAG       1451
Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His Thr Leu Lys
                425                 430                 435
```

FIGURE 1 (CONT.)

```
CAT TTC GAG CAT GTG CGC ATG GTG GAT CCC AAG AAA GCC GCT CAG        1496
His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala Ala Gln
                440                 445                 450

ATC CGG TCC CAG GTT ATG ACA CAC CTC CGT GTG ATT TAT GAG CGC        1541
Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu Arg
                455                 460                 465

ATG AAT CAG TCT CTC TCC CTG CTC TAC AAC GTG CCT GCA GTG GCC        1586
Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
                470                 475                 480

GAG GAG ATT CAG GAT GAA GTT GAT GAG CTG CTT CAG AAA GAG CAA        1631
Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln
                485                 490                 495

AAC TAT TCA GAT GAC GTC TTG GCC AAC ATG ATT AGT GAA CCA AGG        1676
Asn Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg
                500                 505                 510

ATC AGT TAC GGA AAC GAT GCT CTC ATG CCA TCT TTG ACC GAA ACG        1721
Ile Ser Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr
                515                 520                 525

AAA ACC ACC GTG GAG CTC CTT CCC GTG AAT GGA GAG TTC AGC CTG        1766
Lys Thr Thr Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu
                530                 535                 540

GAC GAT CTC CAG CCG TGG CAT TCT TTT GGG GCT GAC TCT GTG CCA        1811
Asp Asp Leu Gln Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro
                545                 550                 555

GCC AAC ACA GAA AAC GAA GTT GAG CCT GTT GAT GCC CGC CCT GCT        1856
Ala Asn Thr Glu Asn Glu Val Glu Pro Val Asp Ala Arg Pro Ala
                560                 565                 570

GCC GAC CGA GGA CTG ACC ACT CGA CCA GGT TCT GGG TTG ACA AAT        1901
Ala Asp Arg Gly Leu Thr Thr Arg Pro Gly Ser Gly Leu Thr Asn
                575                 580                 585

                                        ↓A
ATC AAG ACG GAG GAG ATC TCT GAA GTG AAG ATG GAT GCA GAA TTC        1946
Ile Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp Ala Glu Phe
                590                 595                 600

CGA CAT GAC TCA GGA TAT GAA GTT CAT CAT CAA AAA TTG GTG TTC        1991
Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe
                605                 610                 615

TTT GCA GAA GAT GTG GGT TCA AAC AAA GGT GCA ATC ATT GGA CTC        2036
Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu
                620                 625                 630

                        ↓B
ATG GTG GGC GGT GTT GTC ATA GCG ACA GTG ATC GTC ATC ACC TTG        2081
Met Val Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu
                635                 640                 645
```

FIGURE 1 (CONT.)

```
GTG ATG CTG AAG AAG AAA CAG TAC ACA TCC ATT CAT CAT GGT GTG       2126
Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val
                650                 655                 660

GTG GAG GTT GAC GCC GCT GTC ACC CCA GAG GAG CGC CAC CTG TCC       2171
Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser
                665                 670                 675

AAG ATG CAG CAG AAC GGC TAC GAA AAT CCA ACC TAC AAG TTC TTT       2216
Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe
                680                 685                 690

GAG CAG ATG CAG AAC TAG  2234
Glu Gln Met Gln Asn  *
                695
```

```
        2235  ACCCCC GCCACAGCAG CCTCTGAAGT TGGACAGCAA AACCATTGCT 2280
TCACTACCCA TCGGTGTCCA TTTATAGAAT AATGTGGGAA GAAACAAACC CGTTTTATGA 2340
TTTACTCATT ATCGCCTTTT GACAGCTGTG CTGTAACACA AGTAGATGCC TGAACTTGAA 2400
TTAATCCACA CATCAGTAAT GTATTCTATC TCTCTTTACA TTTTGGTCTC TATACTACAT 2460
TATTAATGGG TTTTGTGTAC TGTAAAGAAT TTAGCTGTAT CAAACTAGTG CATGAATAGA 2520
TTCTCTCCTG ATTATTTATC ACATAGCCCC TTAGCCAGTT GTATATTATT CTTGTGGTTT 2580
GTGACCCAAT TAAGTCCTAC TTTACATATG CTTTAAGAAT CGATGGGGGA TGCTTCATGT 2640
GAACGTGGGA GTTCAGCTGC TTCTCTTGCC TAAGTATTCC TTTCCTGATC ACTATGCATT 2700
TTAAAGTTAA ACATTTTTAA GTATTTCAGA TGCTTTAGAG AGATTTTTTT TCCATGACTG 2760
CATTTTACTG TACAGATTGC TGCTTCTGCT ATATTTGTGA TATAGGAATT AAGAGGATAC 2820
ACACGTTTGT TTCTTCGTGC CTGTTTTATG TGCACACATT AGGCATTGAG ACTTCAAGCT 2880
TTTCTTTTTT TGTCCACGTA TCTTTGGGTC TTTGATAAAG AAAAGAATCC CTGTTCATTG 2940
TAAGCACTTT TACGGGGCGG GTGGGGAGGG GTGCTCTGCT GGTCTTCAAT TACCAAGAAT 3000
TCTCCAAAAC AATTTTCTGC AGGATGATTG TACAGAATCA TTGCTTATGA CATGATCGCT 3060
TTCTACACTG TATTACATAA ATAAATTAAA TAAAATAACC CCGGGCAAGA CTTTTCTTTG 3120
AAGGATGACT ACAGACATTA AATAATCGAA GTAATTTTGG GTGGGGAGAA GAGGCAGATT 3180
CAATTTTCTT TAACCAGTCT GAAGTTTCAT TTATGATACA AAAGAAGATG AAAATGGAAG 3240
TGGCAATATA AGGGGATGAG GAAGGCATGC CTGGACAAAC CCTTCTTTTA AGATGTGTCT 3300
TCAATTTGTA TAAAATGGTG TTTTCATGTA AATAAATACA TTCTTGGAGG AGC         3353
```

FIGURE 2

```
1 GGGGAAGGCC CGGTCATAAC GGCGCTCACG CCC 33

ATG ACG ATC CCC GAT GTG TTT CCG CAC CTG CCG CTC ATC GCC ATC        78
Met Thr Ile Pro Asp Val Phe Pro His Leu Pro Leu Ile Ala Ile
1               5                  10                  15

ACC CGC AAC CCG GTG TTC CCG CGC TTT ATC AAG ATT ATC GAG GTT       123
Thr Arg Asn Prp Val Phe Pro Arg Phe Ile Lys Ile Ile Glu Val
                20                  25                  30

AAA AAT AAG AAG TTG GTT GAG CTG CTG AGA AGG AAA GTT CGT CTC       168
Lys Asn Lys Lys Leu Val Glu Leu Leu Arg Arg Lys Val Arg Leu
                35                  40                  45

GCC CAG CCT TAT GTC GGC GTC TTT CTA AAG AGA GAT GAC AGC AAT       213
Arg Gln Pro Tyr Val Gly Val Phe Leu Lys Arg Asp Asp Ser Asn
                50                  55                  60

GAG TCG GAT GTG GTC GAG AGC CTG GAT GAA ATC TAC CAC ACG GGG       258
Glu Ser Asp Val Val Glu Ser Leu Asp Glu Ile Tyr His Thr Gly
                65                  70                  75

ACG TTT GCC CAG ATC CAT GAG ATG CAG GAC CTT GGG GAC AAG CTG       303
Thr Phe Ala Gln Ile His Glu Met Gln Asp Leu Gly Asp Lys Leu
                80                  85                  90

CGC ATG ATC GTC ATG GGA CAC AGA AGA GTC CAT ATC AGC AGA CAG       348
Arg Met Ile Val Met Gly His Arg Arg Val His Ile Ser Arg Gln
                95                  100                 105

CTG GAG GTG GAG CCC GAG GAG CCG GAG GCG GAG AAC AAG CAC AAG       393
Leu Glu Val Glu Pro Glu Glu Pro Glu Ala Glu Asn Lys His Lys
                110                 115                 120

CCC CGC AGG AAG TCA AAG CGG GGC AAG AAG GAG GCG GAG GAC GAG       438
Pro Arg Arg Lys Ser Lys Arg Gly Lys Lys Glu Ala Glu Asp Glu
                125                 130                 135

CTG AGC GCC AGG CAC CCG GCG GAG CTG GCG ATG GAG CCC ACC CCT       483
Leu Ser Ala Arg His Pro Ala Glu Leu Ala Met Glu Pro Thr Pro
                140                 145                 150

GAG CTC CCG GCT GAG GTG CTC ATG GTG GAG GTA GAG AAC GTT GTC       528
Glu Leu Pro Ala Glu Val Leu Met Val Glu Val Glu Asn Val Val
                155                 160                 165

CAC GAG GAC TTC CAG GTC ACG GAG GAG GTG AAA GCC CTG ACT GCA       573
His Glu Asp Phe Gln Val Thr Glu Glu Val Lys Ala Leu Thr Ala
                170                 175                 180
```

FIGURE 2 (Cont.)

```
GAG ATC GTG AAG ACC ATC CGG GAC ATC ATT GCC TTG AAC CCT CTC      618
Glu Ile Val Lys Thr Ile Arg Asp Ile Ile Ala Leu Asn Pro Leu
                185                 190                 195

TAC AGG GAG TCA GTG CTG CAG ATG ATG CAG GCT GGC CAG CGG GTG      663
Tyr Arg Glu Ser Val Leu Gln Met Met Gln Ala Gly Gln Arg Val
                200                 205                 210

GTG GAC AAC CCC ATC TAC CTG AGC GAC ATG GGC GCC GCG CTC ACC      708
Val Asp Asn Pro Ile Tyr Leu Ser Asp Met Gly Ala Ala Leu Thr
                215                 220                 225

GGG GCC GAG TCC CAT GAG CTG CAG GAC GTC CTG GAA GAG ACC AAT      753
Gly Ala Glu Ser His Glu Leu Gln Asp Val Leu Glu Glu Thr Asn
                230                 235                 240

ATT CCT AAG CGG CTG TAC AAG GCC CTC TCC CTG CTG AAG AAG GAA      798
Ile Pro Lys Arg Leu Tyr Lys Ala Leu Ser Leu Leu Lys Lys Glu
                245                 250                 255

TTT GAA CTG AGC AAG CTG CAG CAG CGC CTG GGG CGG GAG GTG GAG      843
Phe Glu Leu Ser Lys Leu Gln Gln Arg Leu Gly Arg Gle Val Glu
                260                 265                 270

GAG AAG ATC AAG CAG ACC CAC CGT AAG TAC CTG CTG CAG GAG CAG      888
Glu Lys Ile Lys Gln Thr His Arg Lys Tyr Leu Leu Gln Glu Gln
                275                 280                 285

CTA AAG ATC ATC AAG AAG GAG CTG GGC CTG GAG AAG GAC GAC AAG      933
Leu Lys Ile Ile Lys Lys Glu Leu Gly Leu Glu Lys Asp Asp Lys
                290                 295                 300

GAT GCC ATC GAG GAG AAG TTC CGG GAG CGC CTG AAG GAG CTC GTG      978
Asp Ala Ile Glu Glu Lys Phe Arg Glu Arg Leu Lys Glu Leu Val
                305                 310                 315

GTC CCC AAG CAC GTC ATG GAT GTT GTG GAC GAG GAG CTG AGC AAG     1023
Val Pro Lys His Val Met Asp Val Val Asp Glu Glu Leu Ser Lys
                320                 325                 330

CTG GGC CTG CTG GAC AAC CAC TCC TCG GAG TTC AAT GTC ACC CGC     1068
Leu Gly Leu Leu Asp Asn His Ser Ser Glu Phe Asn Val Thr Arg
                335                 340                 345

AAC TAC CTA GAC TGG CTC ACG TCC ATC CCT TGG GGC AAG TAC AGC     1113
Asn Tyr Leu Asp Trp Leu Thr Ser Ile Pro Trp Gly Lys Tyr Ser
                350                 355                 360

AAC GAG AAC CTG GAC CTG GCG CGG GCA CAG GCA GTG CTG GAG GAA     1158
Asn Glu Asn Leu Asp Leu Ala Arg Ala Gln Ala Val Leu Glu Glu
                365                 370                 375
```

FIGURE 2 (Cont.)

```
GAC CAC TAC GGC ATG GAG GAC GTC AAG AAA CGC ATC CTG GAG TTC        1203
Asp His Tyr Gly Met Glu Asp Val Lys Lys Arg Ile Leu Glu Phe
                380                 385                 390

ATT GCC GTT AGC CAG CTC CGC GGC TCC ACC CAG GGC AAG ATC CTC        1248
Ile Ala Val Ser Gln Leu Arg Gly Ser Thr Gln Gly Lys Ile Leu
                395                 400                 405

TGC TTC TAT GGC CCC CCT GGC GTG GGT AAG ACC AGC ATT GCT CGC        1293
Cys Phe Tyr Gly Pro Pro Gly Val Gly Lys Thr Ser Ile Ala Arg
                410                 415                 420

TCC ATC GCC CGC GCC CTG AAC CGA GAG TAC TTC CGC TTC AGC GTC        1338
Ser Ile Ala Arg Ala Leu Asn Arg Glu Tyr Phe Arg Phe Ser Val
                425                 430                 435

GGG GGC ATG ACT GAC GTG GCT GAG ATC AAG GGC CAC AGG CGG ACC        1383
Gly Gly Met Thr Asp Val Ala Glu Ile Lys Gly His Arg Arg Thr
                440                 445                 450

TAC GTG GGC GCC ATG CCC GGG AAG ATC ATC CAG TGT TTG AAG AAG        1428
Tyr Val Gly Ala Met Pro Gly Lys Ile Ile Gln Cys Leu Lys Lys
                455                 460                 465

ACC AAG ACG GAG AAC CCC CTG ATC CTC ATC GAC GAG GTG GAC AAG        1473
Thr Lys Thr Glu Asn Pro Leu Ile Leu Ile Asp Glu Val Asp Lys
                470                 475                 480

ATC GGC CGA GGC TAC CAG GGG GAC CCG TCG TCG GCA CTG CTG GAG        1518
Ile Gly Arg Gly Tyr Gln Gly Asp Pro Ser Ser Ala Leu Leu Glu
                485                 490                 495

CTG CTG GAC CCA GAG CAG AAT GCC AAC TTC CTG GAC CAC TAC CTG        1563
Leu Leu Asp Pro Glu Gln Asn Ala Asn Phe Leu Asp His Tyr Leu
                500                 505                 510

GAC GTG CCC GTG GAC TTG TCC AAG GTG CTG TTC ATC TGC ACG GCC        1608
Asp Val Pro Val Asp Leu Ser Lys Val Leu Phe Ile Cys The Ala
                515                 520                 525

AAC GTC ACG GAC ACC ATC CCC GAG CCG CTG CGA GAC CGT ATG GAG        1653
Asn Val Thr Asp Thr Ile Pro Glu Pro Leu Arg Asp Arg Met Glu
                530                 535                 540

ATG ATC AAC GTG TCA GGC TAC GTG GCC CAG GAG AAG CTG GCC ATT        1698
Met Ile Asn Val Ser Gly Tyr Val Ala Gln Glu Lys Leu Ala Ile
                545                 550                 555

GCG GAG CGC TAC CTG GTG CCC CAG GCT CGC GCC CTG TGT GGC TTG        1743
Ala Glu Arg Tyr Leu Val Pro Gln Ala Arg Ala Leu Cys Gly Leu
                560                 565                 570
```

FIGURE 2 (Cont.)

```
GAT GAG AGC AAG GCC AAG CTG TCA TCG GAC GTG CTG ACG CTG CTC        1788
Asp Glu Ser Lys Ala Lys Leu Ser Ser Asp Val Leu Thr Leu Leu
                575                 580                 585

ATC AAG CAG TAC TGC CGC GAG AGC GGT GTC CGC AAC CTG CAG AAG        1833
Ile Lys Gln Tyr Cys Arg Glu Ser Gly Val Arg Asn Leu Gln Lys
                590                 595                 600

CAA GTG GAG AAG GTG TTA CGG AAA TCG GCC TAC AAG ATT GTC AGC        1878
Gln Val Glu Lys Val Leu Arg Lys Ser Ala Tyr Lys Ile Val Ser
                605                 610                 615

GGC GAG GCC GAG TCC GTG GAG GTG ACG CCC GAG AAC CTG CAG GAC        1923
Gly Glu Ala Glu Ser Val Glu Val Thr Pro Glu Asn Leu Gln Asp
                620                 625                 630

TTC GTG GGG AAG CCC GTG TTC ACC GTG GAG CGC ATG TAT GAC GTG        1968
Phe Val Gly Lys Pro Val Phe Thr Val Glu Arg Met Tyr Asp Val
                635                 640                 645

ACA CCG CCC GGC GTG GTC ATG GGG CTG GCC TGG ACC GCA ATG GGA        2013
Thr Pro Pro Gly Val Val Met Gly Leu Ala Trp Thr Ala Met Gly
                650                 655                 660

GGC TCC ACG CTG TTT GTG GAG ACA TCC CTG AGA CGG CCA CAG GAC        2058
Gly Ser Thr Leu Phe Val Glu Thr Ser Leu Arg Arg Pro Gln Asp
                665                 670                 675

AAG GAT GCC AAG GGT GAC AAG GAT GGC AGC CTG GAG GTG ACA GGC        2103
Lys Asp Ala Lys Gly Asp Lys Asp Gly Ser Leu Glu Val Thr Gly
                680                 685                 690

CAG CTG GGG GAG GTG ATG AAG GAG AGC GCC CGC ATA GCC TAC ACC        2148
Gln Leu Gly Glu Val Met Lys Glu Ser Ala Arg Ile Ala Tyr Thr
                695                 700                 705

TTC GCC AGA GCC TTC CTC ATG CAG CAC GCC CCC GCC AAT GAC TAC        2193
Phe Ala Arg Ala Phe Leu Met Gln His Ala Pro Ala Asn Asp Tyr
                710                 715                 720
```

```
CTG GTG ACC TCA CAC ATC CAC CTG CAT GTG CCC GAG GGC GCC ACC        2338
Leu Val Thr Ser His Ile His Leu His Val Pro Glu Gly Ala Thr
                725                 730                 735

CCC AAG GAC GGC CCA AGC GCA GGC TGC ACC ATC GTC ACG GCC CTG        2283
Pro Lys Asp Gly Pro Ser Ala Gly Cys Thr Ile Val The Ala Leu
                740                 745                 750

CTG TCC CTG GCC ATG GGC AGG CCT GTC CGG CAG AAT CTG GCC ATG        2328
Leu Ser Leu Ala Met Gly Arg Pro Val Arg Gln Asn Leu Ala Met
                755                 760                 765
```

FIGURE 2 (Cont.)

```
ACT GGC GAA GTC TCC CTC ACG GGC AAG ATC CTG CCT GTT GGT GGC        2373
Thr Gly Glu Val Ser Leu Thr Gly Lys Ile Leu Pro Val Gly Gly
                770                 775                 780

ATC AAG GAG AAG ACC ATT GCG GCC AAG CGC GCA GGG GTG ACG TGC        2418
Ile Lys Glu Lys Thr Ile Ala Ala Lys Arg Ala Gly Val Thr Cys
                785                 790                 795


ATC ATC CTG CCA GCC GAG AAC AAG AAG GAC TTC TAC GAC CTG GCA        2463
Ile Ile Leu Pro Ala Glu Asn Lys Lys Asp Phe Tyr Asp Leu Ala
                800                 805                 810

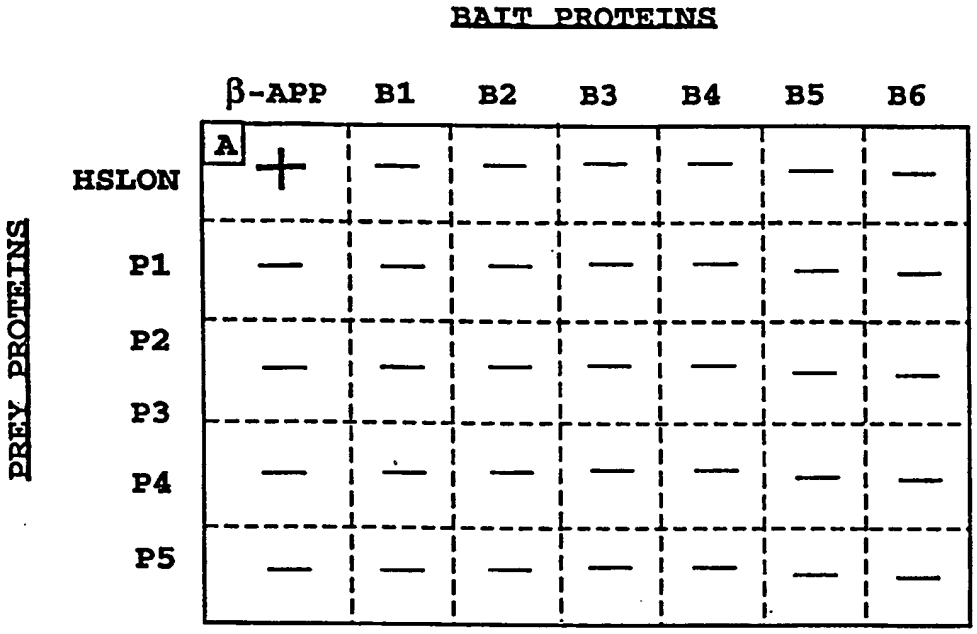
GCC TTC ATC ACC GAG GGC CTG GAG GTG CAC TTC GTG GAA CAC TAC        2508
Ala Phe Ile Thr Glu Gly Leu Glu Val His Phe Val Glu His Tyr
                815                 820                 825

CGG GAG ATC TTC GAC ATC GCC TTC CCG GAC GAG CAG GCA GAG GCG        2553
Arg Glu Ile Phe Asp Ile Ala Phe Pro Asp Glu Gln Ala Glu Ala
                830                 835                 840

CTG GCC GTG GAA CGG TGA          2571
Leu Ala Val Glu Arg *
                845

                                                    2572  CGGCCACCC 2580
CGGGACTGCA GGCGGCGGAT GTCAGGCCCT GTCTGGGCCA GAACTGAGCG CTGTGGGGAG 2640
CGCGCCCGGA CCTGGCAGTG GAGCCACCGA GCGAGCAGCT CGGTCCAGTG ACCCAGATCC 2700
CAGGGACCTC AGTCGGCTTA ATCAGAGTGT GGCATAGAAG CTATTTAATG ATTAAAGTCA 2760
TTTGCAGTGG GAGTTAGCAT CACTAACCTG ACAGTTGTTG CCAGGAATTT GCTTTGTTTA 2820
CTGCTAGTAT ATTAGAAATC CTAGATCTCA GAATCACAAT AGTAATAAAC AACAGGGGTC 2880
ATTTTTTCCT AACTTACTCT GTGTTCAGGT GTGGAATTTC TGTCTCCCAA GAGGAAATGT 2940
GACTTCACTT TGGTGCCAAT GGACAGAAAA TTCTACCTGT GCTACATAGG AGAAGTTTGG 3000
AATGCACTTA ATAGCTGGTT TTTACACCTT GATTTCGAGG TGGAAAGAAA TTGATCATGA 3060
ATCTCTAATA AATTTAAATC TCTTAAACCA AAAAAAAAAA AAA       3103
```

FIGURE 3


BAIT PROTEINS
β-APP
B1
B2
B3
B4
B5
B6
PREY PROTEINS
A
HSLON
P1
P2
P3
P4
P5
+
—